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ABSTRACT

This invention relates to methods for forming multi-lamellar microcapsules of both hydrophilic and hydrophobic immiscible liquid phases using several polymer/solvent systems. Liquid-Liquid diffusion and spontaneous emulsification are controlled by properly timed sequence exposures of immiscible phases in aqueous vehicles dispersed in hydrocarbon solvents containing small quantities of oil, co-surfactants, and glycerides. Water-in-oil and oil-in-water microcapsules are formed containing selected combinations of several types of drugs, co-encapsulated within fluid compartments inside the microcapsule.

Commercial applications of the process and the resultant product relate to drug therapy for treating medical conditions such as cancer, circulatory conditions, and other conditions in which pharmaceuticals are advantageously targeted to specific organs, or delivered in combination with other pharmaceuticals. Small microcapsules may be delivered intravenously to diseased organs or clotted vessels. The use of multiple drugs within the same microcapsule structure provides advantages for applications such as chemoembolization treatments and may be used to deliver both chemotherapeutic drugs, against tumor cells, and an immuno-adjuvant or immunological stimulant to enhance the patient's immune response. Active forms of urokinase and other enzymes may be delivered without dilution to the local site of an embolism for dissolving the embolism. Thus, the invention has several potentially valuable commercial applications related to pharmaceutical and medical applications.

Title: Microcapsules and Methods for Making

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MICROCAPSULES AND METHODS FOR MAKING

BACKGROUND OF THE INVENTION

ORIGIN OF THE INVENTION

The invention described herein may be manufactured and
5 used by or for the Government of the United States of
America for governmental purposes without the payment of any
royalties thereon or therefor.

A. Field of the Invention

The invention relates to methods for making
10 microcapsules, encapsulating pharmaceutical compounds in
microcapsules, microcapsules, microcapsule encapsulated
pharmaceutical compositions and products, and methods of
using the same.

B. Description of the Related Art

15 Many cytotoxic or bioactive drugs and enzymes cannot be
injected intravenously. Others can be injected, but are
rapidly degraded before they reach the target tissue. Still
others are cleared from the blood by the liver or kidneys so
quickly that their biological half-life is too short to be
20 of therapeutic value. Still other drugs are insoluble in
aqueous solutions. Since intravenous injection in
hydrocarbon solvents is not well tolerated by patients, such
drugs are difficult to administer.

One method for overcoming these limitations is
25 encapsulation into microcapsules or liposomes.
Encapsulation of drugs or biological therapeutics into
liposomes or liquid microcapsules can enable delivery to
target organs where the bioactive drug can be released
directly by diffusion. Properly designed microcapsules can

provide unique methods of direct delivery by parenteral injection, nasal inhalation and dermal administration for sustained release of important bioactive drugs.

The size and shape of the microcapsules is critical for the distribution and drug delivery in the tissues. Typically, microcapsules of 1-20 micron diameter are optimum for intravenous administration, whereas, 50-300 micron diameter microcapsules are used for intraarterial delivery and 300 micron or greater for intraperitoneal administration. In each size range, highly uniform microspheres are needed for maximum packing densities and maximum drug payload delivery to target organs or tumors.

Major difficulties with commercial preparation of microspheres arise when density-driven phase separation of the immiscible carrier fluids occurs. This is especially true when the microcapsules are constructed by forming water/oil emulsions or when attempts are made to encapsulate multiple drugs. This limits the yield and often results in microparticles that are not spherical nor uniform in size. Non-conformity limits the packing density (and, thereby, the drug payload delivered) when the microcapsules arrive at the target tissues.

Certain current methods of forming microcapsules (such as liposomes) are based on chemical characteristics of certain phospholipids that self-assemble into bilayers when dispersed in an excess of water. Most liposomes carry pharmaceuticals dissolved in the entrapped water phase. Drugs that are insoluble or that have only limited solubility in aqueous solvents pose problems for incorporating into liposomes. Such organic-soluble drugs are usually limited in liposomal formulations to those that bind inside the hydrophobic region of the liposome bilayer.

Some drugs are so insoluble that they do not associate with the bilayer and, therefore, have very low encapsulation efficiencies. Certain liposomal drug formulations, including anti-tumor liposomes containing dxorubicin
5 [Gabizion et al. 1992] or muramyltripeptide have been studied extensively in clinical trials.

Microcapsule formation by liquid-liquid dispersion of aqueous drugs and organic solvents typically produces water-in-oil (W/O) type liposomes. A second requisite step
10 is removal of the organic solvent (typically evaporated) to form reverse-phase evaporation vesicles (rev) or stable plurilamellar vesicles (splv).

Spherical multilamellar vesicles (mlv) are rarely formed by these methods and the size distribution is quite
15 heterogeneous. Typically, in order to generate multilamellar vesicles, film casting with organic solvents, hydration and sizing using filtration through inert membrane filters is required [Talsma and Crommelin 1992]. Methods of forming multi-layered microcapsules often require
20 emulsification of the aqueous phase into organic carrier solutions by shear, bubbling or sonication. Sophisticated, multi-step emulsion technology is required and yields of uniform type and size are often very low.

Liquid microemulsions also have been developed as drug
25 delivery systems, especially for drugs that are poorly soluble in aqueous carriers. A microemulsion typically contains droplets in the range of 0.1 - 1 μ in diameter. Such microemulsions are characterized by very fluid and dynamic micelles which are formed by sequential mixing one
30 immiscible phase with another using surfactants and co-surfactants [Bhargava et al. 1987]. Typically, surfactants that produce water-in-oil (W/O) microemulsions

have a hydrophilic-lipophilic balance (HLB) rating of 3 to 6, while those that produce oil-in-water (O/W) microemulsions have an HLB of 8 to 18. The surfactants can be non-ionic, ionic, or amphoteric. Often, medium chain-length alcohols are added as the co-surfactant in the last step in achieving the final microemulsion.

The major disadvantages of microemulsions is that each micelle (liquid capsule) is too small (typically, less than 1.0 micron) for deposition in larger vascular beds when administered by intravascular injection. Therefore, microemulsions are not suitable for chemoembolization type treatment of vascularized tumors. Additionally, since microemulsions are true colloidal suspensions, they cannot be scaled up to large enough size for many intravascular drug delivery applications. Microemulsions formed with lipid soluble anti-tumor agents and low density lipoproteins (LDLS) have been used to target drugs to neoplastic cells that require large amounts of cholesterol for synthesis of cell membranes [Halbert et al. 1984]. However, LDLs also attract phagocytes making the amount of drug actually delivered to the tumors and thence the therapeutic dose difficult to determine.

The use of solid matrix microspheres containing adsorbed drugs within the matrix is also known. For instance, U.S. Patent 4,492,720 to Mosier disclosed methods for making microspheres to deliver chemotherapeutic drugs (including Cis-Platinum) to vascularized tumors. This method of preparing microspheres is accomplished by liquid encapsulation and solid-phase entrapment wherein the water-soluble drug is dispersed in a solid matrix material. The method involves dissolving the aqueous drug and the matrix material in a organic solvent, in which they are

mutually soluble, then dispersing this mixture in a second organic solvent to form an emulsion that is stable enough for intravascular injection.

Other approaches have utilized copolymers such as
5 polyvinyl chloride/acrylonitrile dissolved initially in organic solvents to form microparticles containing, for instance aqueous enzyme solutions. U.S. Patent No. 3,639,306 to Sternberg et al. discloses a method of making anisotropic polymer particles having a sponge-like inner
10 support structure comprising large and small void spaces and an outer, microporous polymer film barrier. A multiple-step batch process is used which entails removal of the organic solvents used to dissolve the polymers prior to addition of aqueous components. Solid-matrix microspheres, however, are
15 often not perfect spheres thereby limiting the packing density. Additionally, many drugs cannot be trapped or adsorbed in these systems at effective concentrations and drug-release rates are often not constant.

Conventional methods of forming of multi-lamellar,
20 immiscible, liquid microcapsules are limited, because of density-driven phase separation and stratification into horizontal layers resulting in the necessity to use multi-step, batch processing including mechanical mixing and solvent evaporation phases [Talsma and Crommelin 1992]. Each
25 batch step suffers losses which reduce overall efficiencies. Conventional methods do not permit simultaneous formation of the outer skin as the microcapsule itself is formed. Many conventional therapeutic microspheres have natural phospholipid outer skins (usually in combination with
30 cholesterol and a fatty amine) and therefore are subject to elimination by immune cells. Other conventional methods use sialic acid and other coatings on the lipid bilayer to mask

the liposomes from detection by the scavenging systems of the body. Without an adequate outer skin, microcapsules often coalesce thereby reducing shelf-life.

For instance, U.S. Patent No. 4,855,090 to Wallach,
5 discloses a method of making a multilamellar lipid vesicle by blending an aqueous phase and a nonaqueous lipophilic phase using a high shear producing apparatus. The lipophilic phase is maintained at a high temperature (above the melting point of the lipid components) and is combined
10 with an excess of the aqueous phase, which is also maintained at a high temperature. U.S. Patent No. 5,032,457 to Wallach discloses a paucilamellar lipid vesicle and method of making paucilamellar lipid vesicles (PLV). The method comprises combining a nonaqueous lipophilic phase
15 with an aqueous phase at high temperatures and high shear mixing conditions, wherein the PLVs are rapidly formed in a single step process.

U.S. Pat. No. 4,501,728 to Geho et al. discloses the encapsulation of one or more drugs or other substances
20 within a liposome covered with a sialic acid residue for masking the surface of the membrane from scavenging cells of the body utilizing techniques known for the production of liposomes. In one embodiment, additional tissue specific constituents are added to the surface of the liposome which
25 cause the liposome thusly treated to be attracted to specific tissues. Similarly, U.S. Patent 5,013,556 to Woodle et al. provided methods for making liposomes with enhanced circulation times. Liposomes created by this method contain 1-20 mole% of an amphipathic lipid
30 derivatized with a polyalkylether (such as phosphatidyl ethanolamine derivitized with polyethyleneglycol). U.S. Pat. No. 5,225,212 to Martin et al. discloses a liposome

composition for extended release of a therapeutic compound into the bloodstream, the liposomes being composed of vesicle-forming lipids derivatized with a hydrophilic polymer, wherein the liposome composition is used for
5 extending the period of release of a therapeutic compound such as a polypeptide, injected within the body. Formulations of "stealth" liposomes have been made with lipids that are less detectable by immune cells in an attempt to avoid phagocytosis [Allen et al. 1992]. Still
10 other modifications of lipids (i.e., neutral glycolipids) may be affected in order to produce anti-viral formulations. U.S. Patent 5,192,551 to Willoughby et al. 1993. However, new types of liposomes and microcapsules are needed to exploit the various unique applications of this type of drug
15 delivery.

It is known that microgravity can be advantageously utilized to induce and maintain crystal growth due to the lack of density driven convective flow in liquids. U.S. Patent No. 4,909,933 to Carter et al. discloses an apparatus
20 for carrying out crystallization of proteins and chemical syntheses by liquid-liquid diffusion in microgravity environments. The apparatus comprises a housing having a plurality of chambers and a valve which separates at least two of the chambers so as to allow controlled fluid flow.

25 The disadvantages of conventional liposomes or microcapsules include manufacturing methods that require many batch process steps to: 1) form the liposomes, 2) remove unwanted organic solvents, detergents, and 3) harvest the proper size and shape microparticles for optimum
30 pharmacologic efficacy [Talsma and Crommelin 1992]. Also conventional liposomes often use natural lipids and lectins (from eggs, soybeans and other inexpensive sources) which

attract phagocytic immune cells that rapidly remove the liposomes from the circulatory system before they arrive at the target tissue. This creates variable dose-responses making calculations of pharmacokinetics and therapeutic
5 doses very difficult [Allen 1988]. Major difficulties with commercial preparation of microcapsules often involves density-driven phase separation of the immiscible carrier fluids, esp. when forming water/oil systems.

These drawbacks limit the yield, make it difficult to
10 harvest the proper size particle, and often result in micro-particles that are not spherical nor uniform in size, thereby limiting the packing density (and drug payload delivered) when the microcapsules arrive at the arterioles or capillaries in the target issues. Liposomes have a
15 bilayer outer membrane which requires that the entrapped drug must be soluble in both the aqueous and lipid phases in order to outwardly diffuse. This limits the type of drugs that can be released from conventional liposomes and the mole ratio of aqueous to lipid phases limits the amount of
20 drug which can be delivered.

Processes are needed for forming spherical multi-lamellar microcapsules having alternating hydrophilic and hydrophobic liquid layers, surrounded by flexible, semi-permeable hydrophobic or hydrophilic outer membranes
25 which can be tailored specifically to control the diffusion rate. In particular, methods of making such microcapsules are needed which do not rely on batch processes involving density-driven phase separation and stratification into horizontal layers, mechanical mixing and solvent evaporation
30 phases. Moreover, there is clearly a need for methods and compositions which allow for uniform size and more spherical microcapsules. Such improved microcapsules would be

particularly useful in the delivery of pharmaceutical compositions.

SUMMARY OF THE INVENTION

Processes and compositions are provided by the present invention which overcome certain of the limitations of prior methodology for forming microcapsules. In particular, methods and compositions are provided which form multi-lamellar microcapsules having alternating hydrophilic and hydrophobic liquid layers, surrounded by flexible, semi-permeable hydrophobic, outer membranes which can be tailored specifically to control the diffusion rate. In particular, the methods of making microcapsules provided by the present invention do not rely on batch processes such as density-driven phase separation and stratification into horizontal layers, mechanical mixing or solvent evaporation. Encapsulation of cytotoxic or labile drugs in such microcapsules enables targeted delivery and sustained release kinetics that are not currently available with intravenous injection.

The invention provides, in one aspect, methods of making a multi-layered microcapsule. The term microsphere as used herein is a general term which can include any spherical microscopic vesicle including microspheres, micelles, inverted micelles, bilayer vesicles and liposomes. The term microcapsule as used herein is a more specific term which refers to a microsphere which comprises at least two layers, one of which is innermost and is substantially completely enclosed within the other. In a distinct break from traditional methods for making microcapsules, the methods of the invention rely on low shear mixing and liquid-liquid diffusion process, particularly as developed

for forming microcapsules that may contain both aqueous and hydrocarbon soluble drugs.

The terms multi-layered and multi-lamellar are used interchangeably throughout the specification and claims and
5 both refer to the fact that the microcapsules of the invention comprise at least two immiscible layers nested around one another. In some instances, the core layer will be hydrophobic in nature and will be completely surrounded by at least one neighboring hydrophilic layer. In others,
10 the core layer will be hydrophilic in nature and will be completely surrounded by at least one neighboring hydrophobic layer.

The basic method of the invention relies on liquid-liquid interactions. In the basic method, the first step
15 entails formulating a first phase or layer while the second step entails formulating a second phase or layer. The two phases or layers are formulated to be immiscible with one another. For the purposes of this invention, "immiscible" means that the solubility of one phase or layer is not more
20 than 10gm/100ml in an adjoining phase or layer and that the two adjoining phases or layers form an interface resembling a meniscus.

Formulating the first phases or layer comprises combining a first solvent, a first polymer soluble in the
25 first phase, a co-solvent, an oil, and water. The first solvent will typically comprise about 75-90% by volume of the first phase. The first polymer is selected to be one soluble in the first phase and typically will comprise about 1-5% by volume of the first phase. A small amount of a co-
30 solvent is also added to the first phase, which co-solvent may also function as a co-surfactant. Oil comprising about

1-10% by volume is also added to the formulation. The first phase will also contain about 1-5% water by volume.

The method next calls for formulating a second phase immiscible with the first phase. The second phase comprises
5 a second solvent, a second polymer soluble in the second phase, a surface active agent, and a salt. The relative, approximate volume percentages of these constituents is about 70-98% second solvent, 1-10% second polymer, 1-4% surface active agent, and 1-3% salt.

10 In order to ensure that the liquid-liquid interactions necessary to form the microcapsule will occur, certain of the constituents of each phase are selected relative to one another. Thus, the surface active agent in the second phase is selected such that it will have a hydrophilic/lipophilic
15 balance value greater than that of the first polymer constituent of the first phase. Generally, the most useful surface active agents have been found to be those which are nonionic and which have a hydrophilic/lipophilic balance value of 10.0 or greater. Next, the second polymer
20 constituent of the second phase is selected to have a hydrophilic/lipophilic balance value lower than that of the surface active agent constituent of the same phase. While not an exhaustive list, certain hydrophilic/lipophilic balance values of materials which may be used in the
25 formulations of the invention are provided below.

HYDROPHILIC/LIPOPHILIC BALANCE (HLB) (McCutcheon 1979)

<u>Compound</u>	<u>HLB</u>
Glycerol treideate	0.8
Cholesterol	1.0
30 Triglyceride of coconut oil	1.4
Sorbitan tiroleate	1.8

	<u>Compound</u>	<u>HLB</u>
	Sorbitan tristearate	2.1
	Glycerol monooleate	2.7
	Mono and di glycerides of fat burning fatty acids	2.8
5	Glycerol Monostearate (gms)	2.8-5.0 (3.8 preferred)
	Propoxylated ethylene diamine plus ethylene oxide	3 - 28
	Mono/diglyceride	3.2
	Glycerol mono coconut	3.4
10	Mono/diglyceride	3.5
	Propylene glycol mono fatty acid ester	3.5
	Monoethoxyl lauryl ether	3.6
	Stearyl lactyl acid	3.8
	Hydrogenated cottonseed oil	3.8
15	Sodium lauryl sulfate	4.0
	Mono and diglycerides with citric acid or lactic ester or fatty acid	4.2-4.6
	Ethoxylated fatty amine (2 moles ETO)	4.5
20	Diethylene glycol monostearate	4.7
	Sorbitan monopalmitate	4.7
	Diethylene glycol monostearate and oleate	4.7
	Ethoxylated (2) cetyl ether	5.3
25	Glycerol Monoricinoleate	6.4
	Glycerol monolaurate	6.8
	Triglycerol mono stearate	7.0
	Polyethylene glycol (400 dioleate)	7.2
	Lanolin sterol	8.0

	<u>Compound</u>	<u>HLB</u>
	Ethoxylated nonyl phenol (CO-420 & CO 850)	8.0-16.0
	Polyethylene glycol (400) distearate	8.2
	Sorbitan monolaurate	8.6
5	Ethoxylated sorbitan fatty acid esters and alkyl/aryl alcohol	9.0
	Anhydrous lanolin	10.0
	Polyethylene glycol monostearate	11.0
	Polyethylene glycol 400	11.2
10	Ethoxylated (10) cetyl ether	12.9
	Ethoxylated glycerol monostearate (gms)	13.1
	Sorbitan monostearate	14.9
	Sorbitan monooleate with 20 moles ethylene oxide	15.0
15	Ethoxylated (20) oleyl ether	15.3
	Ethoxylated (20) stearyl cetyl ether	15.8
	Ethoxylated castor oil	18.0
	Nonyl phenol polyethylene glycol ether	18.1
	Polyethylene glycol 600 mono laurate	19.6
20	Sodium lauryl sulfate	40
	Propylene glycol monostearate	40
	Hydroxylated lanolin sodium oleyl sulfate	42
25	Blends of GMS and sorbitan monooleate with 20 mols ethylene oxide	52

The basic method next involves creating an interface between the first and second phases. The creation of the interface is achieved in such a way that minimal shear and mixing occurs between the phases. The two immiscible phases are brought together in such a mechanical manner that the

fluid shear properties are controlled to low levels, below about 12 dynes/cm², and such that the adsorptive surface properties at the immiscible interfaces are not significantly altered. Although the exact mechanisms are not fully understood, the inventors believe that the maintenance of certain surface properties, such as the surface tension, Helmholtz charge distribution (electrical double layer), and partitioning of the surfactant molecules between the immiscible phases must remain substantially intact so that lateral phase separation can occur in a manner which allows simultaneous formation of multiple liquid interfaces (oil/water or water/oil) and which results in microcapsules having alternating spherical shells of hydrophilic and hydrophobic liquid layers. This is believed to be the mechanism for the formation of multi-lamellar vesicles which are formed in a single step. Although this can best be demonstrated under microgravity conditions, wherein buoyant convection is absent and diffusion-driven convection predominates, this also can be accomplished in unit gravity conditions by balancing the density differences between the two liquid phases or by any other mechanical means which prevents excess fluid shear from significantly altering the normal adsorptive surface properties which are determined by the chemical composition of the formulas and the interfacial phenomena among the solvents, polymers and surfactants. In a preferred embodiment, the creation of the interface will occur by sliding individually separated compartments containing the two phases into register with one another in a manner that substantially limits shear and provides gentle mixing.

In the final step of the basic method, conditions are established in order to substantially limit all mixing between the interfaced liquid phases. In the most preferred environment, the two phases would be allowed to interact at their interface without agitation, stirring, shearing or like force. It is preferred to also limit even those quiescent forces such as gravity-controlled sedimenting, shifting, drift and the like. Thus, in certain preferred embodiments, only chiefly diffusion-driven convection is used to spontaneously form microcapsules, as the chemical formulations of the different phases assist in lowering the surface free energy across the interface. It is also at this time that formation of the polymeric outer coating is initiated.

In one embodiment, the two liquids thus formulated are separated into distinct compartments or spaces which spaces are each connected to a central diffusion chamber into which each compartment can deliver its resident liquid loading. The compartments are initially closed to access into the central diffusion chamber so that the first and second liquids are kept apart from one another and not allowed to interact. While it is possible to use any number of devices to achieve this separation, a preferred device is a device like the Materials Dispersion Apparatus described in more detail below. The separation of the two liquids is maintained until both liquids and the device containing them can be placed in an environment in which convective mixing may be minimized, such as in a microgravity environment.

The methods of the invention are slightly different depending upon whether the first solvent is selected to be organic or aqueous. Where an organic solvent is used to

formulate the first phase, that organic solvent is selected from the group of organic solvents consisting of ethyl alcohol, methyl alcohol and isopropyl alcohol. Where an organic first solvent is used to formulate the first phase, 5 the first polymer is selected to be one soluble in the organic solvent selected. Such a first polymer may be selected from the group of polymers consisting of glycerol monostearate, glycerol monooleate, glycerol monolaurate, glycerol dioleate, glycerol distearate, cholesterol, 10 stigmasterol, phytosterol, campesterol, and lecithins such as phosphatidyl cholines (e.g., Centrox-F™).

Where the first solvent is aqueous, a slightly different approach is taken. In those instances, the first polymer is again requisitely soluble in the first aqueous 15 phase and may be selected from the group of polymers consisting of polyvinyl pyrrolidone, polyvinyl alcohols, gelatin, gum tragacanth, carrageenan, Karaya gum, Guar gum, gum arabic, alginates, carboxymethyl cellulose, hydroxypropyl cellulose, carboxypropyl cellulose, and 20 lecithins.

Regardless of the formulation with an aqueous or organic first solvent and polymer, the methods of the invention both use a co-solvent which may be selected from the group of co-solvents consisting of C₃-C₈ alcohols, 25 tetrahydrofuran, dioxane, acetonitrile, dimethylformamide, dimethylacetamide, and dimethyl sulfoxide. Similarly regardless of the organic/aqueous nature of the first solvent and polymer used, the methods of the invention add to the formulation of the first phase an oil. These oils 30 may be selected from the group of oils consisting of unsaturated oils such as poppy seed oil, olive oil, peanut

oil, sesame oil, cotton seed oil, soybean oil, safflower oil, corn oil, sunflower seed oil and canola oil or saturated oils such as mineral oil, long chain paraffinic oil, and liquid petrolatum. In a preferred embodiment, 5 poppy seed oil will be iodinated to form iodinated poppy seed oil (IPO) and incorporated into a microcapsule as a marker or tracer for tracking the presence of the microcapsule once injected via radiocontrast detection methods known well to those of skill in the art of 10 radiography.

Whether the method involves an organic or an aqueous first solvent, the second polymer, the surface active agent and the salt may each be selected from a particular group of such compounds. The second polymer may be selected from the 15 group of polymers consisting of polyethyleneglycol 1000-8000 daltons, dextran 1000-10000 daltons, polyvinylpyrrolidone, polyvinyl alcohols, gelatin, gum tragacanth, carrageenan, Karaya gum, Guar gum, gum arabic, alginates, carboxymethyl cellulose, hydroxypropyl cellulose, carboxypropyl cellulose, 20 and lecithins. The surface active agent is selected from the group of surface active agents consisting of sorbitan monooleate treated with ethylene oxide, dextran, polyethylene glycol, C₁₂-C₂₀ fatty acids, 2-amino-2-methyl-1-propyl aminomethyl propanol amphoteric salts and quaternary 25 ammonium salts. The salt is selected from the group of salts consisting of NaCl, KCl, CaCl₂, MgCl₂, quaternary ammonium salts, cetyl trimethylammonium bromide and 4-methoxy-4(3-phosphatidyl choline)spiro(1,2-dioxetane-3-g,1-adamantane) disodium salt.

30 In certain embodiments of the methods of the invention, pharmaceutical compositions will be incorporated into the

microcapsule. Where such pharmaceuticals are thusly incorporated, they may be introduced initially as a solute or as particulates suspended in one or the other of the liquids used to formulate the layers of the microcapsules.

5 In certain embodiments, the pharmaceutical is introduced in one of the phases or layers used to produce the microcapsule at a concentration sufficient to allow nascent crystal formation within said microcapsule. Crystal formation may occur at or near the time of formation of the microcapsule

10 containing the dissolved pharmaceutical material. The aqueous solvent system used to dissolve an aqueous-soluble pharmaceutical is selected to permit water molecules to migrate away from the drug-containing layer into the alcoholic mixture. The process of crystal formation is

15 likely to be promoted in this manner after formation of the microcapsule. In fact, it is possible to enhance the crystallization process after the microcapsule is formed by controlled transport of the solvent phase or layer in which the pharmaceutical to be crystallized is a solute. It

20 certain embodiments, the crystal thus formed may take up most of the internal capacity of the microcapsule.

Multi-layered microcapsules, with both hydrophobic and hydrophilic drug compartments, as produced by the methods of the invention enable diffusion of complimentary drugs from

25 the same microcapsule, e.g. antibiotics and immuno-stimulants to treat resistant infections or multiple fibrinolytic drugs to dissolve emboli. Co-encapsulation of radio-contrast medium as provided herein enables oncologists to monitor the delivery of anti-tumor microcapsules to

30 target tumors using computerized tomography and radiography that track the distribution of microcapsules after release

from the intra-arterial catheter. Such microcapsules will have important applications in chemotherapy of certain liver, kidney, brain and other tumors.

5 The diameters of microcapsules possible to attain using the methods of the invention are also of particular usefulness in medical applications. Thus, whereas prior art methods have been able to routinely produce microspheres over 1-10 micron average sizes, the present invention's methods provide similarly-sized microcapsules of 1-20 micron
10 diameters for intravenous administration. Also provided are 50-300 micron sized microcapsules particularly useful in interarterial chemoembolization of tumors, and microcapsules in the range of 300 micron and greater diameters useful in interperitoneal administered drugs.

15 The pharmaceutical composition encapsulated in the microcapsule may be one soluble in aqueous solutions or may be one soluble in organic solutions. This, of course, governs the selection of the phase or layer in which the pharmaceutical composition is formulated. The microcapsules
20 of the invention and methods for producing them are of particular utility when formulating organic-soluble drugs as these type of drugs are otherwise very difficult to administer. The pharmaceuticals may be those selected from the group of such widely diversified pharmaceutical
25 compositions as that consisting of cytotoxins, proteases, cytokines, anti-nauseants, steroids, anti-fungal agents, fibrinolytic enzymes, and antibiotics. The inventors have successfully encapsulated representatives of these classes of pharmaceuticals using the methods of the invention. It
30 is also possible to incorporate a pharmaceutical composition which is not initially dissolved in one or another of the

phases or layers, but rather which drug is in suspension. As noted above, depending upon its solubility and upon where the pharmaceutical chemist wishes to locate the drug, it is possible to formulate a drug in any of the phases or layers, 5 by dissolving or suspending the drug as needed.

The methods of the invention surprisingly demonstrated the ability to package very high concentrations of drugs in the layers formed. It is possible, using the methods of the invention, to formulate a pharmaceutical at a concentration 10 sufficient to allow nascent crystal formation within the microcapsule once it is formed. These microcapsules, due in one regard to their being constructed with outer polymeric coatings, are also particularly flexible yet rugged (able to withstand shear forces greater than 10 dynes/cm²). As will 15 be related specifically below, microgravity experiments, on sounding rockets (1989-92) and Shuttle missions STS-52 (1992) and STS-56 (1993) using an automated Materials Dispersion Apparatus, produced multi-lamellar microcapsules containing both Cis-platinum (anti-tumor drug) and iodinated 20 poppy seed oil (a non-radioactive, radiocontrast medium), surrounded by a polymeric skin. Microcapsules formed with amoxicillin (antibiotic) or urokinase (a clot dissolving enzyme), co-encapsulated with IPO, were still intact after two years after return to 1 x g environments. In many 25 instances, microcapsules were formed with the Cis-Platinum or amoxicillin so concentrated that crystals of the drugs formed inside.

Surprisingly, the methods of the invention have demonstrated a unique ability to encapsulate such saturated 30 drug solutions, and since the overall partitioning characteristics between immiscible layers facilitates

solvent transport out of the aqueous layer, it is possible to concentrate the drug to the point that formation of drug crystals occurs within the microcapsules. This ability of the microcapsules and methods of the invention provides the maximum drug payload per microcapsule and the best drug release kinetics for prolonged treatment at maximum drug diffusion rates.

Microcapsules containing a large volume component of crystalline drug provide the most concentrated drug possible when it is released at the target site. Until the crystals are completely dissolved, the drug release rate is independent of time (zero order release kinetics). When the crystals have dissolved, the drug release rates revert to first order kinetics (exponential with time). The encapsulated crystals of the invention are in the range of 1-50 microns in diameter. Since these crystals are precipitated in situ, they are quite different from the other commercially-available crystalline drug delivery systems (e.g., Microcrystal™) which use phospholipids to encapsulate tiny particles or crystals of drugs with an average diameter of only 0.3 - 1.0 micron [Parikh and Stern 1994].

It is also possible to additionally treat the microcapsules thus formed with additional steps. In some instances, the methods of the invention, regardless of whether they initially use an organic or an aqueous first solvent, formulate a third phase comprising an oil or C₂₀-C₃₈ paraffin and, contact the formed microcapsule with the third phase. In other instances, the methods of the invention form a two-layered microcapsule, then formulate a third phase comprising an aqueous solution and, contact the formed

microcapsule with the third phase. The basic method and alternatives are summarized below.

	Group 1	Group 2
Solution 1	<p><u>Solvent 1</u> is a hydrocarbon</p> <p><u>Polymers</u> are hydrocarbon soluble, selected to form the outer coating (typically of lower HLB values)</p> <p><u>Co-solvents</u> alcohols, hydrocarbons (act as co-surfactants)</p> <p><u>Oils</u> saturated or unsaturated oils</p> <p><u>Drug</u> dissolved (or suspended particulate)</p>	<p><u>Solvent 1</u> is aqueous</p> <p><u>Polymers</u> (skin) are water soluble, but can be extended into organic phase (includes phospholipids) Ex. Centrolex F™</p> <p><u>Co-solvents</u> same, but often less %</p> <p><u>Oils</u> same</p> <p><u>Drug</u> dissolved (or particulate)</p>
Solution 2	<p><u>Solvent 2</u> aqueous</p> <p><u>Polymers</u> water soluble (PEG, Dextran)</p> <p><u>Surfactants</u> (typically higher HLB value)</p> <p><u>Salts</u> ionic, quaternary ammonium salts</p> <p><u>Drugs</u> aqueous soluble</p>	<p><u>Solvent 2</u> same</p> <p><u>Polymers</u> same</p> <p><u>Surfactants</u> same but often less %</p> <p><u>Salts</u> same, but often different %</p> <p><u>Drugs</u> aqueous soluble</p>
Solution 3	<p><u>Oils</u> hydrocarbons</p> <p><u>Polymers</u> hydrocarbon-soluble</p> <p><u>Drugs</u> can be included</p> <p style="text-align: center;">-- OR --</p> <p><u>Alternative</u> aqueous solution</p> <p style="padding-left: 40px;"><u>coating</u>-adjuvants immunoglobulins <u>polymers</u> - aqueous soluble <u>surfactants</u> -</p>	<p><u>Oils</u> same</p> <p><u>Polymers</u> same</p> <p><u>Drugs</u> can be included</p> <p style="text-align: center;">-- OR --</p> <p><u>Alternative</u> aqueous solution</p> <p style="padding-left: 40px;"><u>coating</u> same <u>polymer</u> same <u>surfactants</u> same</p>

Traditional emulsion methods form a O/W/O (oil/water/oil) or W/O/W (water/oil/water) liquid system which is designed to retain the internal phase(s) within the external solvent unless the emulsion is broken, whereupon
5 the liquid phases separate. In the methods of the invention, the use of surfactants and co-surfactants permits formation of an emulsion of large spheroids (not small microspheroids) of one phase dispersed in the other phase configured in a sphere. The sphere is also surrounded by
10 another immiscible liquid layer (opposite phase to that of the innermost liquid sphere) and then (often) this multi-layered sphere is contained in another opposite-phase liquid layer and finally the entire multi-layered sphere is contained in an outer skin. The results of the process of
15 the invention are not to form a traditional O/W/O or W/O/W emulsion (which is a fine dispersion of one phase in another), but rather to form multi-lamellar, alternating immiscible-layer microcapsules contained within a thin, semi-permeable outer skin. In the microcapsules of the
20 invention, the immiscible phases are distinct and separated according to the surface tension characteristics of the liquids at each interface, hence there is no true emulsion maintained by the surfactant which could be broken.

Thus, in certain embodiments of the methods and
25 compositions of the invention, the multi-layered microcapsule will be produced which comprises at least three alternating layers or phases. Thus, if the first layer is an aqueous layer or core, the next layer may be an organic layer. This organic layer may then be covered over by a
30 second aqueous layer which forms on its outer surface a polymeric skin. Conversely, the liquid at the core of the

microcapsule may be an organic liquid layered over by an aqueous layer followed by another organic layer which forms a polymeric skin over the surface of the microcapsule. Certainly, extension of these basic formulations may be
5 envisioned where four or more layers are possible or where multiple skins or coatings are utilized.

Whether used in conjunction with a two-layer microcapsule or with microcapsules with more than two layers, the coatings of the present invention are of
10 substantial utility, particularly when the methods are carried out at earth-normal gravity. The coatings can be either substantially of a hydrophobic nature or of a hydrophilic nature as described below and are derived from addition of certain polymers in the initial formulations of
15 the liquids used to make the microcapsules. Where hydrophobic coatings are used in conjunction with drug-delivery systems, the coatings are selected for their complementary permeability to the drug to be delivered. The polymers are also selected for their flexible
20 characteristics after formation and curing which is of particular utility during intravascular transport and allows higher packing densities for forming emboli such as in chemoembolization therapy. Thus, for example where a water-soluble drug is to be delivered, the drug is contained in an
25 inner aqueous layer over which is placed a coating permeable to the dissolved drug. Preferably, the coating material should be impermeable to solvents or oils. The coatings which have been observed to be deposited on the surfaces of the microcapsules of the invention are about 0.01-2.0
30 microns thick where the coating is a hydrophobic coating,

and about 0.1-5.0 microns thick where hydrophilic coatings are deposited.

The additional steps and third formulated phases may also be used advantageously to provide the microcapsule with specific characteristics. Thus, the third phase may further
5 comprise a pharmaceutical composition which is added to the formed surface of the microcapsule. The third phase may also be used to add a pharmaceutical composition such as an adjuvant. The adjuvant may further comprise an
10 immunoglobulin, other protein, hydrocolloid or polysaccharide. This is of particular utility in designing microcapsules with unique immunologic, proteinaceous or other surface characteristics which makes them selectively adhere to certain target tissues (cells) or renders the
15 microcapsules attractive to certain phagocytic cells (when the cells are the actual target for the therapeutic drug). Where the adjuvant is a hydrocolloid, it may be selected from the group of such hydrocolloids consisting of collagen, isoelectric gelatin, agar, gum arabic, gum tragacanth,
20 alginates, cellulose derivatives and carrageenans. The third phase may also further comprise a surface active agent.

The third aqueous phase can also contain a chemical activator which acts upon the inactive form of the
25 pharmaceutical agent (drug) as it diffuses out of the inner layers of the microcapsule. The function of the activator is to chemically convert the inactive drug to its active form just before it is released from the microcapsule. This is illustrated when the pharmaceutical is a pro-enzyme and
30 where the activator is another proteolytic enzyme which cleaves the pro-enzyme at active site to render the molecule

biologically active. This embodiment can be used to deliver very labile drugs which have very limited shelf-lives or short biological half-lives whereupon the activator (third phase) can be added shortly prior to intravascular
5 administration such that the inactive drug becomes activated after the microcapsules have reached the target site. This can maximize the therapeutic effectiveness of the short-lived drug at the target site of action.

One or more of the phases of the microcapsule of the
10 invention may further comprise fluorescent molecules selected from the group of fluorescent molecules consisting of fluoresceins, cyanins, naturally fluorescent molecules, and rhodamines. This is particularly useful where radiocontrast media are not desirable or where an additional
15 tracking method is useful or where it is of value to monitor the presence or absence of a layer in the microcapsule, fluorescent molecules may be incorporated into the microcapsule of the invention. Thus, for instance, as described more fully below, it may be useful to incorporate
20 a hydrophilic fluorescent molecule in the aqueous liquid in order to determine the relative location and number of aqueous liquid layers in a certain production batch of microcapsules produced by the methods of the invention.

Critical to the success of the methods of the invention
25 is the substantial limitation of mixing between said phases to diffusion-driven convection. One manner in which to so limit other types of mixing is to carry out the methods under microgravity. Microgravity is defined as a gravity force of less than $1 \times 10^{-3} \times g$. Such gravitational
30 environments may be achieved in a variety of ways, at least some of which are detailed herein. For instance,

microgravity may be achieved in certain trajectories of sounding rockets. Even longer periods of microgravity may be obtained with temporary orbiters such as the space shuttle. Relatively indefinite periods of microgravity may
5 be obtained in permanent or semipermanent orbital space craft such as the orbital space station and other geosynchronous orbital satellites. The exposure of the first and second liquids to microgravity has been found to be effective in forming the microcapsules of the invention
10 where the exposure is at least 6.5 minutes in duration. Certainly, as described more fully below, greater exposure periods have also been proven successful. The inventors anticipate that periods of exposure as short as 1.0 minute will also produce adequate numbers of microcapsules.

15 In preferred embodiments, however, the methods of the invention will not use microgravity in order to limit mixing between the phases. Of course, such limitations of mixing can be promoted by carrying out the methods below ambient temperature. Limitation of interactions between the phases
20 is best promoted by substantially balancing the specific gravity between said phases as is described below. The formulations and methods necessary to achieve earth-normal microcapsule formation are described in greater detail herein. In either case, or in combinations of these
25 techniques, mixing between the two phases may be chiefly the result of diffusion-driven convection.

The inventors have found that there is a greater size distribution which results from microencapsulation at earth-normal gravity. At least a partial reason for this wider
30 size distribution is apparently the inability under earth-normal gravity to avoid certain sedimentation phenomena

alone and sedimentation effects combined with weight-related contact of sedimented microcapsules. These facts require some additional manipulation under earth-normal environments not required in the 0 x g environments -- namely, sieving of
5 the resulting microcapsules in order to generate more uniform fractions. Therefore, at earth normal gravity, the utility of the outer coating of the microcapsules of the present invention become even more important. Enhancing the ruggedness of the earth-normal microcapsules by curing and
10 other steps as related herein may also be used.

A preferred method of making a multi-layered microcapsule comprises: formulating a first phase comprising an organic solvent selected from the group of organic solvents consisting of ethyl alcohol, methyl alcohol and
15 isopropyl alcohol, a first polymer soluble in the first phase selected from the group of polymers consisting of glycerol monostearate, glycerol monooleate, glycerol monolaurate, glycerol dioleate, glycerol distearate, cholesterol, stigmasterol, phytosterol, campesterol,
20 lecithins such as phosphatidyl cholines (e.g., Centrox-F[™]), a co-solvent selected from the group of co-solvents consisting of C₃-C₈ alcohols, tetrahydrofuran, dioxane, acetonitrile, dimethylformamide, dimethylacetamide, and dimethyl sulfoxide, an oil selected from the group of oils
25 consisting of poppy seed oil, olive oil, peanut oil, sesame oil, cotton seed oil, soybean oil, safflower oil, corn oil, sunflower seed oil, canola oil (unsaturated oils), or mineral oil, long chain paraffinic oil, and liquid petrolatum (saturated oils), and water; formulating a second
30 phase immiscible with the first phase, the second phase comprising water, a second polymer soluble in the second

phase selected from the group of polymers consisting of polyethyleneglycol 1000-8000 daltons, dextran 1000-10000 daltons, polyvinylpyrrolidone, polyvinyl alcohols, gelatin, gum tragacanth, carrageenan, Karaya gum, Guar gum, gum
5 arabic, alginates, carboxymethyl cellulose, hydroxypropyl cellulose, carboxypropyl cellulose, lecithins, a surface active agent selected from the group consisting of sorbitan monooleate treated with ethylene oxide, dextran, polyethylene glycol, C₁₂-C₂₀ fatty acids, quaternary ammonium
10 salts, and a salt selected from the group of salts consisting of NaCl, KCl, CaCl₂, MgCl₂, quaternary ammonium salts, cetyl trimethylammonium bromide, 2-amino-2-methyl-1-propyl aminomethyl propanol, and 4-methoxy-4(3-phosphatidyl choline)spiro(1,2-dioxetane-3-g,1-adamantane) disodium salt;
15 the surface active agent having a hydrophilic/lipophilic balance value greater than that of the first polymer; the second polymer having a hydrophilic/lipophilic balance value lower than that of the surface active agent; creating an interface between the first and second phases in a manner
20 that substantially limits fluid shear; and, substantially maintaining adsorptive surface characteristics of said interface.

Microcapsule products produced by any of the methods of the invention are also claimed. The methods of the
25 invention are used to form unique multi-lamellar, microcapsules, having alternating hydrophilic and hydrophobic liquid layers surrounded by a flexible, semi-permeable, polymeric outer "skin". The outer skin which can be either hydrophilic or hydrophobic, is designed to
30 allow controlled drug diffusion out of the microcapsule.

Unlike any natural phospholipid or other component of cell membranes, the outer skin of the microcapsules of the invention avoids recognition and phagocytosis by immune cells, thereby increasing the amount of drug delivered to the tissues. The multi-layered microcapsules of the invention can entrap multiple drugs in different solvent compartments and saturated solutions of drugs which may then form crystals inside the microcapsule. Radiocontrast medium can be co-encapsulated with drugs in the same microcapsule. A magnetic resonance contrast agent can also be encapsulated such as various metallo-organic compounds including aqueous soluble ferrous gluconate, Gadolinium diethylene triamine pentaacetic acid and hydrocarbon-soluble, iron pentacarbonyl.

The microcapsules of the invention have been found to provide a surprisingly uniform distribution of diameters (Fig. 3A and B). This uniformity is particularly important in its medical applications. The microcapsules thus produced can be used to deliver several drugs which can be released sequentially to the target tissues. The deformable, liquid-filled microcapsules also have advantages over solid matrix microspheres in achieving maximum packing density in blood vessels, thereby decreasing blood flow to target tissues. This enhances the therapeutic effect of combined drug delivery and reducing the blood supply to vascular tumors (chemoembolization).

The methods of the invention result in more spherical, uniform size distributions of microcapsules. When comparing certain prior art equipment and methods for forming microcapsules (Microfluidics, Inc., see Figs. 2A, B and 3A, B), the inventors found that even the preferred formulations

of the invention were incapable of providing such
uniformity. In certain instances, hardly any microcapsules
formed at all where mixing and vortexing were used to
distribute one phase into the next (Fig. 2A). In others,
5 poorly formed and non-spherical microcapsules resulted (Fig.
2B). In contrast to the failures of the prior art methods,
the methods of the invention were successfully used to
generate uniform, spherical microcapsules both under unit
gravity (Fig. 3A) and under microgravity conditions (Fig.
10 3B). Such uniformity enables superior drug delivery.
Enhanced uniformity also enables better dose distribution
calculations for establishing the therapeutic dose in the
treatment of specific diseases, especially treatment of
certain types of tumors. Importantly, the methods of the
15 invention allow the formation of larger-sized,
multi-lamellar microcapsules (1-350 micron) than heretofore
possible. Such a capability allows multilamellar
microcapsules to be made specifically for inhalation and
deposition in the lungs. This uniformity allows facile
20 sieving or filtering of the microcapsule products in order
to obtain highly uniform diameter fractions.

Most liposomes have a very small hydrophobic
compartment and therefore can only carry small amounts of
hydrophobic drugs. Contrastingly, the microcapsules of the
25 invention have a relatively large hydrophobic liquid
compartment which enables delivery of more hydrophobic drug
per microcapsule. Moreover, the microcapsules of the
invention have relatively large hydrophilic and hydrophobic
compartments which permits tandem delivery of both water
30 soluble and non-water soluble drugs in the same
microcapsule.

As previously noted, the microcapsules of the invention may contain polysaccharides. Inclusion of such polysaccharides is one of several aspects of the methods of the invention that enhance the formation of the

5 microcapsules. The inclusion of injectable polysaccharides in the formulations of the invention (similar to those polysaccharides found in Ringer's solutions) contributes to the driving forces that control phase separation and phase partitioning of the entrapped drugs. The polysaccharides

10 also provide increased shelf-life and stability of the parenteral suspensions. Use of the osmotically neutral salt solutions in the aqueous phase enhances micelle formation, lateral phase separation, and increases the dispersion of microcapsules and their stability as they are formed.

15 The methods of the invention in a preferred embodiment utilize a non-phospholipid outer coating. The microcapsules formed by this method are contained in a thin, semi-permeable, outer membrane comprised of hydrophobic (e.g. mono- or polyglycerides or waxy-polymers) or

20 hydrophilic polymers (e.g., PVA or PVP), depending on the desired diffusion release rate of the encapsulated drug. Thus, the coating has the advantage of allowing design of the appropriate drug diffusion and release characteristics while avoiding certain of the disadvantages of conventional

25 liposomes (and lipid bilayers). In particular, the coating produced by the methods of the invention around the outer surface of the microcapsule avoids being readily detected and largely eliminated by the reticuloendothelial system (RES). The outer skin protects the microcapsules against

30 shear forces encountered during manufacturing processes and during transport within the vascular system enroute to the

target tissues. The hydrophobic outer membrane also can be designed to retard oxygen transport, thereby reducing oxidative degradation of the entrapped drug and improving the shelf-life of the parenteral suspensions. The

5 flexible/deformable outer skin on the microcapsules of the invention results in increased packing densities within vascular beds. This results in microcapsules superior to solid microspheres (e.g. gelatin, albumin or starch) commonly used for chemoembolization therapy against tumors.

10 The formulations used to produce the microcapsules of the invention are summarized below.

Formulas for Primary, Secondary and Tertiary Solutions
for Microencapsulation

Group 1

<p><u>Primary Solution</u> (also can contain drug)</p>	<p><u>Secondary Solution</u></p>	<p><u>Tertiary Solution</u> (also can contain dissolved drug)</p>
<p><u>First Solvent</u> (75-90%) ethyl alcohol methyl alcohol isopropyl alcohol</p> <p><u>Organic Co-solvent</u> 0-20% C₄-C₈ alcohols tetrahydrofuran (THF) dioxane acetonitrile dimethylformamide (DMF) dimethyl sulfoxide (DMSO)</p> <p><u>Polymers</u> (1-5%) (monoglycerated) glycerol monostearate glycerol monooleate glycerol monolaurate</p> <p>(polyglycerides) glycerol dioleate glycerol distearate</p> <p>(sterols) cholesterol plant sterols - stigmasterol phytosterol campesterol</p> <p>(phospholipids) lecithins e.g., phosphatidyl choline (Centrolax-F[™])</p>	<p><u>Second Solvent</u> water (70-98%)</p> <p><u>Polymers</u> (1-10%) polyethylene glycol PEG - 1000-8000</p> <p>(polysaccharides) Dextran 4000-20000 (range 10000-100000))</p> <p>others polyvinyl-pyrrolidone polyvinyl alcohols</p> <p><u>Surfactants</u> (ionic and non-ionic) (1-4%) sorbitan monooleate plus ethylene oxides Dextran PEG C₁₂-C₂₀ fatty acid quaternary NH₄ salt</p>	<p><u>Oils</u> (up to 100%) IPO heavy mineral oil olive oil same as in primary soln. paraffins (C₂₀-C₃₈)</p> <p><u>Alternative</u> Aqueous solutions containing - immunoglobulins albumin gelatin hydrocolloids plant sterols phospholipids polysaccharides - starches - cyclodextrins</p> <p><u>Polymers</u></p> <p><u>Surfactants</u> (1-4%) (ionic and non-ionic) long chain amphoteric salts celluloses</p>

<p align="center"><u>Primary Solution</u> (also can contain drug)</p>	<p align="center"><u>Secondary Solution</u></p>	<p align="center"><u>Tertiary Solution</u> (also can contain dissolved drug)</p>
<p><u>Water</u> (1-5%) water</p> <p><u>Oils</u> (unsaturated or saturated) (1-10%)</p> <ul style="list-style-type: none"> iodinated poppy seed oil (IPO) mineral oil cotton seed oil olive oil safflower oil canola oil peanut oil sesame oil corn oil <p><u>Dissolved Drugs</u> (1% to saturation)</p>	<p><u>Additional Polymers</u> (1-10%) (hydrocolloids)</p> <ul style="list-style-type: none"> gelatin gum tragacanth carrageenans karaya gum guar gum alginates <p>(celluloses)</p> <ul style="list-style-type: none"> celluloses (CMC, WEC, HPC) <p><u>Salts</u> (1-3%)</p> <ul style="list-style-type: none"> NaCl KCl, CaCl₂, quaternary NH₄ salts, cetyl trimethylammonium bromide, 2M2A-AMP, PPD <p><u>Dissolved Drugs</u> (1% to saturation) therapeutic of choice</p>	<p><u>Additional Polymers</u> Same as secondary solution</p> <p><u>Dissolved Drugs</u> (1% to saturation) soluble therapeutic</p>

Table 1 (cont'd)

	<u>Primary Solution</u> (also can contain drug)	<u>Secondary Solution</u>	<u>Tertiary Solution</u> (also can contain dissolved drug)
Group 2	<p><u>Aqueous First Solvent</u> water (70-90%)</p> <p><u>Co-solvents</u> (0-20%) C₃-C₈ alcohols tetrahydrofuran (THF) dioxane acetonitrile dimethylformamide (DMF) dimethyl sulfoxide (DMSO)</p> <p><u>Polymers</u> hydrophilic (water soluble) polyvinylpyrrolidone (PVP) polyvinyl alcohols (PVA)</p> <p><u>hydrocolloids</u> gelatin gum tragacanth carrageenans karaya gum guar gum</p> <p><u>alginates</u> <u>celluloses</u> CMC, CPC <u>phospholipids</u> lecithins phosphatidyl choline Centrox F</p> <p><u>polysaccharides</u> corn starch cyclodextrins</p> <p><u>Oils</u> (unsaturated or saturated) 1-10% iodinated poppy seed oil (IPO) mineral oil cotton seed oil olive oil safflower oil canola oil peanut oil sesame oil corn oil</p> <p><u>Dissolved Drugs</u> 1% to saturation</p>	<p>Same as Group 1</p> <p><u>Co-Solvents</u> Same as primary solution</p> <p><u>Polymers</u> (1-10%) Same as Group 1</p> <p><u>Surfactants</u> (1-20%) (ionic and non-ionic) Same as Group 1</p> <p><u>Additional Polymers</u> 1-10%</p> <p><u>Salts</u> (1-3%) Same as Group 1</p> <p><u>Dissolved Drugs</u> 1% to saturation</p>	<p><u>Oils</u> (up to 100%) Same as Group 1</p> <p><u>Alternatives</u> Aqueous solutions Same as Group 1</p> <p><u>Polymers</u> Same as Secondary Solution</p> <p><u>Surfactants</u> Same as Secondary Solution</p> <p><u>Dissolved Drugs</u> 1% to saturation</p>

The formulations of the invention, in preferred
embodiments, are less toxic than conventional liposomes and
other microcapsules by avoiding use of certain phospholipids
and long chain amines (eg. sterylamine) contained in
5 conventional microspheres which can produce toxic side
effects.

Where the microcapsules of the invention comprise a
pharmaceutical composition, certain medically related
advantages may be obtained. Thus, due to the uniformity and
10 ease with which the methods of the invention allow formation
of multilamellar microcapsules, co-encapsulation of multiple
drugs is made possible. Thus, for instance, as will be
described more fully below, co-encapsulation of drugs and
radiocontrast medium in the same microcapsules is made
15 possible by the methods of the invention. Such co-
encapsulation allows radiological monitoring of the tissue
distribution during intravascular delivery. Additionally,
incorporation of fluorescent-labels for entrapped drugs
enables accurate measure of the drug compartment volumes
20 (using fluorescent imaging techniques) and convenient
determinations of the drug loading efficiencies, particle
size distributions and measurement of shelf-life stability
of the final parenteral suspensions. In some applications
made possible by the methods and compositions of the
25 invention, the organic phase can include a tracer compound
or radiocontrast medium to provide the additional advantage
of real-time imaging of the microcapsules with computerized
tomography (CT) scanning as they are released from the
catheter enroute to the target tissue. Other examples
30 include aqueous soluble metallo-organic compounds used for
diagnostic imaging such as ferrous gluconate or Gadolinium

diethylene triamine pentaacetic acid (Gd-DTPA) used for nuclear magnetic resonance imaging and hydrocarbon soluble agents such as iron pentacarbonyl which also may be used for NMR imaging.

5 Production of multi-layered microcapsules via the methods of the invention which possess alternating hydrophobic and hydrophilic drug compartments allows for design of multiple-therapy microcapsules. Spontaneous formation of microcapsules with one or more large
10 hydrophobic solvent compartments increases the potential application for delivery of more aqueous-insoluble drug at target sites with adequate vascular networks. By using the microcapsules made possible by the methods of the invention, sequential diffusion of two or more drugs out of the same
15 microcapsule may be achieved at the target tissues. The incorporation of aqueous-soluble cyclodextrin which can act as an internal hydrophobic drug carrier is also made practical using the single step methods and formulations provided in this invention. This extends the capability of
20 the invention in delivering otherwise aqueous-insoluble drugs.

For instance, the use of multiple drugs within the same microcapsule provides microcapsules specifically designed for chemoembolization treatments. Multiple-drug
25 microcapsules also may be used to deliver first a chemotherapeutic drug which kills tumor cells, and then an immuno-adjuvant (tumor necrosis factor) or immunological stimulant (e.g. interferon-g) that would enhance the patient's immune response to the tumor. Multiple-drug
30 microcapsules can also be used to deliver combinations of chemotherapeutic drugs to tumors that are located in

privileged sites, such as brain tumors. For example, and as described more fully in the examples to follow, simultaneous delivery of different types of drugs in the same microcapsule is made possible with the methods and compositions of the invention, e.g. diaziquone and cis-platinum to brain tumors via the carotid artery [Kimier et al. 1993]. Multi-layered microcapsules may also be used to treat deep infections that are resistant to systemic antibiotics. In these applications, one or more antibiotics may be sequentially delivered to the site of the infection. Multi-layered microcapsules can be designed to protect active forms of urokinase and other thrombolytic enzymes until they are delivered and entrapped at the local site of a blood clot, where therapeutic doses of the enzyme may then diffuse out to dissolve the unwanted embolism. The multilamellar microcapsules can also be used to deliver immunostimulants; cytokines such as Interferons, Interleukins, and growth factors; antinauseants such as metoclopramide and tetrahydrocannabinol; multiple fibrinolytic enzymes such as urokinase (uPA), tissue plasminogen activator (tPA) and streptokinase; steroids such as hydrocortisone, dexamethasone, etc.; anti-fungals such as nystatin and griseofulvin, anti-virals such as amatidine, iododeoxuridine, riboviran; and multiple antibiotics such as amoxicillin, ampicillin, etc.

In one embodiment, as related to the space-based research that lead to the earth-normal embodiments of the invention, exposure to microgravity for at least 1.0 minutes in duration is accomplished. If the microcapsules of the invention are to be used in 1xg environments, as is generally anticipated, an additional step comprising

recovering the multi-layer microcapsules will be necessarily accomplished at earth normal gravity. Generally, this step will be accomplished by reentry and recovery of the orbital device by which exposure to 0xg was accomplished. While it is preferred to accomplish the recovery without exposure of the formed microcapsules to physical extremes (pressure, temperature, shearing, mixing, etc.), recovery of the microcapsules of the invention have been accomplished via a transition from microgravity to earth normal gravity at accelerations of at least 15xg without substantial loss of integrity.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Conceptual schematic showing formation of a multi-lamellar microcapsule with an aqueous drug/oil dispersion at its center, a hydrocarbon/oil drug #2 and/or radiocontrast medium (e.g. IPO) as a next layer, aqueous layer/drug (cis-platinum) as a next layer, and a polymer outer skin.

Fig. 2. Photomicrographs of prior art method (vigorous mixing) to make microcapsules. Fig. 2A shows a typical result which fails to form microcapsules. Fig. 2B depicts certain best efforts to form microcapsules using prior art method.

Fig. 3. Photomicrographs depicting the distribution of sizes of microcapsules resulting from the methods of the invention are applied to form microcapsules. Fig. 3A shows the typical result when the methods used under unit gravity. Fig. 3B shows similar results when microcapsules are formed using the microgravity methods of the invention.

Fig. 4. Photomicrograph at enhanced magnification over that of Fig. 3A and B showing details of certain microcapsules of the invention.

Fig. 5. Photomicrographs of a microcapsule of the invention which was made with a fluorescent dye. Fig. 5A shows a single microcapsule without using fluorescence microscopy. Fig. 5B shows the same microcapsule as photographed using fluorescence microscopy.

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Fig. 6. Microcapsules with crystalline structures in their internal layers or shells. Fig. 6A shows a microcapsule with a single cubic crystal of Cis-Platinum trapped within. Fig. 6B shows a microcapsule with numerous crystals of Cis-
5 Platinum formed within.

Fig. 7. Microcapsules which have been treated to contain a radio-contrast oil (iodinated poppy seed oil) and crystals of Cis-Platinum.

DESCRIPTION OF PREFERRED EMBODIMENTS

A series of more than 38 separate experiments on four space flights has led to the development of this invention. These experiments along with their ground-based counterparts are described below for the purpose of pointing out the invention specifically and providing details useful in carrying out the invention. These specific examples, however, do not limit the scope of the claimed invention.

Referring first to the figures, Fig. 1 is a conceptual schematic showing formation of a multi-lamellar microcapsule **10** with an aqueous drug/oil dispersion **12** at its center **14**, a hydrocarbon/oil drug#2 and/or radiocontrast medium (e.g. IPO) **16** as a next layer **18**, an aqueous layer/drug (cis-platinum) **20** as a next layer **22**, and a polymer outer skin **24**. A first hydrocarbon phase **26** and a second aqueous phase **28**, initially separated, are allowed to form an interface **21** with minimal mixing and low shear. If only diffusion-driven convection is allowed to occur at interface **21** thereafter, microcapsules of the invention form representative of that depicted **10**.

Fig. 2 is a pair of photomicrographs of the resulting mixture when a prior art method (utilizing vigorous mixing) is used with the preferred formulations of the invention to attempt to make microcapsules at earth normal gravity. Fig. 2A shows a typical result which fails to form microcapsules of any kind. Fig. 2B depicts certain best efforts to form microcapsules using this method. In Fig. 2B, it can be seen that certain poorly formed microcapsules **30** have formed. Typically, these microcapsules will demonstrate considerable

lack of sphericity **32**, coalescence **34**, and non-uniformity **36**.

In Fig. 3, a pair of photomicrographs of the resulting mixture of when the methods of the invention are applied to form microcapsules. Fig. 3A shows the typical result when the methods of the invention are used to form microcapsules under unit gravity, at temperatures below ambient. Numerous microcapsules wherein the first phase is an organic phase formed, including certain ones of considerable diameter **40** as well as those of fairly small diameter **42**. Fig. 3B shows similar results when microcapsules are formed using the microgravity methods of the invention using a first aqueous phase. Again, numerous microcapsules, including certain ones of considerable diameter **44** as well as those of fairly small diameter **46** form. It is clear from such photomicrographs, that uniformity and sphericity is a common characteristic of the microcapsules of the invention, regardless of the gravity environment.

Fig. 4 is a photomicrograph at enhanced magnification over that of Fig. 3A and B showing details of certain microcapsules of the invention when practiced at unit gravity. A single large microcapsule **50** and several smaller ones **52** are shown. Nested inside microcapsule **50** can be seen several oil solvent spheroids (olive oil) **51** each of which is surrounded by the inner aqueous layer **54**. A outer polymeric coating **56** can be seen on microcapsules **50** and **52**. It is also possible to detect 3-4 spherical shells **58** nested within one another on certain of the smaller microcapsules **60**.

Fig. 5 is a photomicrograph of a microcapsule formed under microgravity of the invention which was made with a

fluorescent dye in order to demonstrate the ability of the microcapsules of the invention to segregate a drug into a distinct layer. Fig. 5A shows a single microcapsule 70 in focus without using fluorescence microscopy comprising a
5 outermost polymeric coating 72, an internal hydrocarbon solvent phase 74, a second polymeric membrane 76, aqueous spheroids 78 contained within the hydrocarbon inner layer 74, and an aqueous shell layer 80. Fig. 5B shows the result when the same microcapsule was photographed using a light
10 source and optics to enable visualization of the fluorescent dye location, the internal spheroids 78 and aqueous shell or layer 80 are seen to fluoresce to indicate the location of the dye 82 therein.

Fig. 6 depicts the capacity of the methods of the
15 invention to create spherical microcapsules of uniform and substantial volumes which are capable of forming crystalline structures in their internal layers or shells. Fig. 6A shows a microcapsule 90 with a single cubic crystal of Cis-platinum 92 trapped within. Fig. 6B shows a microcapsule 94
20 with numerous crystals of Cis-Platinum 96 formed within.

Fig. 7 shows a collection of microcapsules 110 of the invention which have been treated to contain a radio-contrast oil (iodinated poppy seed oil) 100 and crystals of Cis-Platinum 102 contained in microcapsules 104 with uniform
25 spherical shells 106 and an outer polymeric coating 108.

Example I: MICROGRAVITY EXPERIMENTS SUMMARY

The basic formulations and simplified liquid-liquid, dispersion methods were developed in 1988 and 1989. The conceptual approach is shown in Fig. 1. Fig. 1 is a
30 schematic showing formation of a multi-lamellar microcapsule

with an aqueous drug/oil dispersion at its center, a hydrocarbon/oil drug#2 and/or radiocontrast medium (e.g. IPO) as a next layer, an aqueous layer/drug (e.g., cis-platinum) as a next layer, and a polymeric outer coating or skin. Microencapsulation-related experiments designed to overcome the limitations of the first methods were conducted on six space missions beginning in April 1989 with the Consort-I sounding rocket using the Materials Dispersion Apparatus (MDA) mini-lab developed by Instrumentation Technology Associates, Inc. The sounding rocket flights produced only 6.5 minutes of microgravity conditions, but this was adequate to form the unique microcapsules in a single step. Experiments on the Space Shuttle permitted 10 minute dispersion times followed by curing of the outer polyglyceride skin for eight days under microgravity conditions. A summary of these experiments is shown in Table 2. New formulations were tested on Shuttle STS-52, using only aqueous-soluble drugs, polymers and surfactants, and on STS-56 using alcohols as co-surfactants. The specific experiments and results are described in detail in the examples to follow.

Table 2. MED Flight Experiments Summary

MISSION	DATE	EXPERIMENTS	MATERIALS	RESULTS
Consort-1	4/89	protein diffusion	urokinase & antibodies	diffusion rates established
Consort-1	3/90	diffusion kinetics	urokinase & myoglobin	kinetics verified
Consort-4	11/91	microencapsulation of drugs ^{ab}	Cis-Platinum, amoxicillin, urokinase & Strept-avidin	multi-lamellar microspheres w/ alternating hydrophilic & hydrophobic layers
Consort-5	9/92	microencapsulation of drugs ^{ab}	Cis-platinum, amoxicillin & urokinase	multi-lamellar microspheres w/ alternating hydrophilic & hydrophobic layers
STS-52	10/92	microencapsulation of drugs (aqueous polymers only) ^{ab}	Cis-platinum, amoxicillin & urokinase	multi-lamellar microspheres, crystals within microcapsules
STS-56	4/93	microencapsulation of drugs (alcohol co-surfactants) ^{ab}	Cis-platinum, amoxicillin & urokinase	multi-lamellar microspheres, crystals within microcapsules

^a Fluorescent labels included ^b Fluorescent beads included

Example II: GRAVITY-DEPENDENT RESTRICTIONS RECOGNIZED

Gravity-dependent restrictions in the basic liquid-liquid spontaneous microencapsulation process led to the design of several microgravity experiments to explore the utility of this process when density-driven phenomena were eliminated. In particular, density-driven, gravity-dependent restrictions of the liquid-liquid microencapsulation process were: early phase separation producing fragile microcapsules; interfacial dynamic flow causing coalescence of microcapsules. Failure of ground-based experiments to derive uniform microcapsules lead to a desire to attempt microcapsule formation in space.

The microgravity flight experiments led to the development of a new liquid-liquid microencapsulation process that involves use of surfactants and co-surfactants in the aqueous phase and co-surfactant alcohols in the organic phase, which also contained, in one embodiment, high molecular weight polymers that formed a tough outer "skin" on the final microcapsules. In microgravity, a single step dispersion produced unique multi-lamellar microcapsules containing various aqueous drugs co-encapsulated with iodinated poppy seed oil (a radiocontrast medium with a sp. gravity =1.35). Subsequent ground control experiments also produced some of these unique microcapsules and illustrated that the 1 x g process could be improved to yield useable microcapsules by using different formulations. In particular, it became clear that the outer coatings substantially improved the ruggedness of the microcapsules formed.

Example III: SOUNDING ROCKET EXPERIMENTS

Initial experiments on Consort-1 and -3 were used to determine the effective mixing and diffusion kinetics in the MDAs (see below for apparatus description). This showed
5 that sufficient volume was mixed at the interface via diffusion to allow formation of microcapsules. These experiments also provided the diffusion constants for each of the components of the liquid phases.

The first successful microencapsulation of drugs in
10 microgravity was conducted on the Consort-4 mission in November 1991. The microcapsules were recovered and analyzed by microscopic image analysis. Mono-dispersed fluorescent beads were included as internal size standards and fluorescent labels were used to determine the
15 distribution of drug in the various fluid compartments. Additional experiments, conducted on Consort-5 in September 1992, confirmed the capabilities of the new method for forming multi-lamellar microcapsules with alternating layers of hydrophilic and hydrophobic drugs.

20 Microcapsules formed in 38 microgravity mini-experiments used liquid-liquid dispersion of aqueous drug solutions, surfactant and polyethylene glycol dispersed in alcoholic co-surfactant solutions containing soluble polyglycerides.

25 Microcapsules of both oil/water and polymer/water/oil were recovered from the Consort flights. These experiments produced multi-lamellar liquid microcapsules (concentric spheres within spheres) comprised of three or more, alternating immiscible layers. Image analysis of the
30 microcapsules was made possible by co-encapsulation of standard size fluorescent beads. Microcapsules were formed

in the ranges of 1-15 microns, 40-50 microns, 110-130 microns and 160-230 microns in diameters. This was a substantial improvement over the prior art approaches which had initially been attempted by the inventors to derive
5 microcapsules only in the 10 micron and less range. The size distribution covered a range of from about as low as 5 microns in diameter up to about 300 microns in diameter and greater. The average size of the microcapsules formed in
10 these experiments was about 150 microns, greatly in excess of the average 10 micron or less diameters obtained with prior art approaches.

The ruggedness of the microcapsules formed under these conditions allowed for size segregation by sieving. Digital analysis (National Institutes of Health image analysis
15 program) of phase contrast and fluorescent images taken with a fluorescent microscope also confirmed that the aqueous-soluble drugs were routinely encapsulated within the inner aqueous core and the outermost aqueous shell of the microcapsules.

20 This typical distribution is illustrated in Fig. 5, which is a composite of a transmitted light photomicrograph and a fluorescent photomicrograph (lower right) of the same multi-lamellar microcapsule. The polyglyceride skin is clearly shown in the normal-light photomicrograph (upper
25 left).

Multi-lamellar microcapsules were, also formed which contained relatively large amounts of IPO (Guerhart Laboratories - France, Savage Laboratories - U.S.A.) in discrete lamella. Fig. 7 shows a microcapsule heavily
30 loaded with IPO, which often comprised up to 38% of the total volume. Often small hemispheres of IPO were also

found clinging to the outer surface of the large inner (aqueous) sphere or adhered to the outer polymer skin of the microcapsule.

Microcapsules formed by almost all of the formulations survived 15+g accelerations, severe vibrations and turbulent mixing, during the reentry of the experiment capsule, and have remained intact for two years after recovery from space. These multi-layered microcapsules are similar to liquid-filled, thin-skinned, micro-balloons which are flexible enough to be manipulated on a microscope slide without collapse.

The microcapsules formed in just 6.5 minutes of microgravity retain their spherical shape and appear tough enough to survive the extensive physical manipulations required for sizing, final preparation and storage of parenteral suspensions, and the fluid shear encountered after intravascular injection.

Also formed were very unusual structures (multiple small spheres of aqueous-soluble drug) distributed within multi-lamellar o/w/o microcapsules, wherein the aqueous spheroids are arranged in an annular ring that appears fixed in a plane within the innermost sphere (not shown). These ring structures remain intact when the microcapsules are "rolled around" on the microscope slide without rupturing. These structures demonstrate the ability of the methods of the invention to form small spheroids that do not coalesce inside the larger microcapsule. Such structures may be advantageously used to control the specific volume to surface area ratio in order to control the rate of diffusion of a solute in such spheroids. In particular, sustained

release of pharmaceuticals contained in such spheroids within microcapsules may find utility.

Example IV: SPACE SHUTTLE EXPERIMENTS

Microencapsulation experiments on Consort 4 and Consort
5 5 used mixtures of aqueous-soluble drugs, IPO, C3-C8
alcohols and polyglycerides that are insoluble in aqueous
solutions. In experiments conducted on STS-52, the inventors
co-encapsulated cis-platinum (diaminodichlor-cis-platinum;
Bristol Laboratories) with IPO by forming microcapsules from
10 water-soluble polymers using special formulations of
aqueous, non-alcoholic solvents. Such formulations will
find particular utility in co-encapsulations of anti-tumor
compounds along with radiocontrast medium for tracking drugs
in the body.

15 Polyvinyl pyrrolidone (PVP) and a commercial lethicin
(Centrox-F™; a lecithins compound produced by U.S. Soya,
Inc.) were used to form multi-lamellar microcapsules at 20
°C. Fluorescent beads and fluorescent labeled were co--
encapsulated with the drugs to permit drug-distribution
20 measurements, within the various lamellae, using
fluorescence microscopy and digital image analysis at the
NASA Johnson Space Center, Houston, Texas. The final
microcapsules were suspended and recovered in either aqueous
solutions, IPO or mineral oil. The microcapsules formed by
25 these formulations were similar to those made using
alcohol-soluble polyglycerides. However, without the
hydrocarbon-soluble polyglyceride skin these microcapsules
were more fragile.

Another unique type of microcapsule was formed during
30 these experiments that was characterized by drug crystals

formed within the inner aqueous core of the multi-lamellar microcapsules. Fig. 6B shows an example of a microcapsule which is packed (approximately 65% of the aqueous compartment) with crystals of Cis-platinum, an anti-tumor
5 drug. Microcapsules containing crystals of amoxicillin were also formed in the STS-52 experiments (not shown). These illustrate that aqueous-soluble drugs can be encapsulated at very high concentrations near the solubility limit of the drug. After the microcapsules are formed the drug can become
10 further concentrated (perhaps via the alcohol absorbing the water from the aqueous phase in which the pharmaceutical solute is dissolved) to form large crystals which are more stable than the dissolved drug during prolonged storage.

Microcapsules formed from first organic solvent/polymer
15 methods appeared to be more rugged (by visual comparison under the microscope) than those formed on STS-52 formed from first solvent aqueous/polymer methods. The STS-56 experiments again produced multi-lamellar liquid microcapsules (multiple concentric spheres within spheres)
20 comprised of alternating immiscible layers. Using fluorescent 6.4 micron beads and image analysis, it was found that the most interesting microcapsules were formed in the range of 10-15 micron, 40-50 micron, 50-100 micron, and 160-230 micron diameters. These diameter distributions were
25 of particular interest since it is known that intraarterial uses can accommodate 50-300 micron diameter microcapsules while intravenous applications can only tolerate 1-10 micron microcapsules. Thus, by segregating the microcapsules into sized fractions (sieving), it should be possible to address
30 particular intravascular limitations.

As noted above, microcapsules were formed containing crystals of cis-Platinum or amoxicillin. The crystals apparently were formed after encapsulation. Several microcapsules were formed that contained a single, large
5 cubic crystal of Cis-Platinum which so completely filled the inner sphere that only about 15% of the inner volume remained as a liquid. One encapsulated, cubic Cis-Platinum crystal was measured at 48 μ across within a 57 μ diameter microcapsule (similar to that shown in Fig. 6A). After
10 formation, some of the microcapsules were dispersed in an external oil phase (either IPO or mineral oil) and allowed to cure for eight days before return to Earth.

These microgravity experiments have shown that formation of multi-lamellar, alternating-phase microcapsules
15 can be controlled by proper timed-sequence exposures of the immiscible phases using special solvent formulations and surfactants. Once formed, these microcapsules remain spherical due to the predominant surface tension of the internal phases and polymer/solvent phase partitioning at
20 the interfaces.

These experiments clearly demonstrated the capability to use liquid-liquid diffusion mixing to form unique microcapsules containing hydrophilic and hydrophobic drugs under microgravity conditions. Thus, ground-based
25 experiments were conducted to compliment and replicate the space experiments. These ground-based experiments were able to replicate the size range (5-250 microns in diameter) to a limited degree, but the average size microcapsule obtained was about 10-40 microns in diameter. Still, this was a
30 substantial improvement over the prior art approaches which rarely formed microcapsules over 10 microns in diameter. It

was also observed that the ground-based experiments resulted in less rugged microcapsules. This is likely a result of the gravity-dependent deformations of the spherical microcapsules as they form giving rise to areas of thinner
5 polymer deposition. Thus, the flexible microcapsules, formed under microgravity conditions, have more uniform size distributions than those formed in 1 x g, are more rugged, and have a higher average diameter than ground-made
10 microcapsules, largely due to the absence of thermal convection, buoyancy forces, and instabilities that occur at the immiscible interfaces.

The microgravity experiments illustrate the feasibility of co-encapsulating aqueous-soluble drugs, hydrocarbon-soluble drugs and oil-based contrast media
15 within a lipid-soluble, polyglyceride outer film which cures rapidly enough to be impervious to oil or hydrocarbon resolubilization. They also allow the formation and harvesting of unique microcapsules which are durable enough to be removed from the external solvent without disruption
20 or destruction of the internal phases. It is anticipated that these microcapsules will have several advantages over conventional liposomes that are designed for intravascular injection.

Example v: FLIGHT HARDWARE DESCRIPTION

25 The microencapsulation experiments described herein were conducted using the Materials Dispersion Apparatus (MDA; ITA, Inc., Exton, PA). The MDA's consist of an upper and a lower block that contain chambers for each sample fluid. The blocks are misaligned at launch so that the
30 chambers are not in contact with each other. Upon activation

in microgravity, the blocks are moved to align the chambers so that the fluids can mix by liquid-liquid diffusion. Some of the experiments were conducted with a single-step fluid mixing, and some were done with a two-step fluid mixing
5 technique which allows diffusion of a third fluid or sample into the mixture of the first two fluids while still in the microgravity environment.

Example VI: DISCUSSION AND ALTERNATIVE EMBODIMENTS

Spontaneous formation of multi-lamellar, microcapsules
10 containing alternating layers of aqueous and hydrophobic solvent compartments is strongly dependent on the interfacial tension and the amount of mixing between immiscible liquid phases. On Earth this process is limited by gravity-dependent, density-driven separation of the
15 immiscible liquids into stratified horizontal layers. In microgravity, this process is largely dependent on the surface-free energies of the different liquids, but independent of density-driven convection or buoyant phase separation. Hydrocarbon soluble, high molecular weight
20 polymers have been included in the formulations to form flexible, permeable "skins" or outer coatings around the liquid microcapsules as they are created by phase partitioning mechanisms. It is also possible to form such polymer barriers between internal layers. The microcapsules
25 can be formed and cured without deformation by contact with container walls.

More specifically, co-encapsulation of an aqueous-soluble, anti-tumor drug (Cis-platinum) and a radio-contrast medium (IPO), in microgravity, has produced a
30 unique drug delivery system that can be visualized by

radiologic or computerized tomography scanning to insure that the cytotoxic drug is delivered directly to the target tumor. Multi-layered microcapsules have been developed which can provide a new intravascular delivery system for 5 targeted tissues and sequential, sustained release of multiple anti-tumor drugs. This method has resulted in formation of flexible spherical microcapsules of more uniform sizes, which can provide maximum packing densities and maximum drug delivery to target organs or tumors.

10 Multi-layered microcapsules can be designed to protect active forms of urokinase and other thrombolytic enzymes until they are delivered and entrapped at the local site of a blood clot, where therapeutic doses of the enzyme can diffuse out to dissolve the unwanted embolism. These 15 immiscible-liquid diffusion methods also could be used for encapsulating certain labile drugs to make microcapsules for special purpose drug delivery systems, especially those designed to deliver drugs via the nasal or buccal mucosa or via inhalation directly to the lungs. Examples include 20 protected delivery of mucolytic DNase for sustained release treatment of cystic fibrosis and α anti-trypsin for patients with deficiencies in the lung epithelium.

Example VIII: REDISPERSION OF MICROCAPSULES IN AQUEOUS OR OIL VEHICLES

25 A frequently used second step includes dispersion of the microcapsules (after they have formed) in different aqueous/polymer solvents or in a pure oil phase. A unique attribute of microcapsules formed by these methods is that they do not re-dissolve in an oily external phase, even 30 though the semi-permeable outer skin is hydrophobic. This

produces a suspension in the liquid carriers that are commonly used for intravascular administration.

Example IX: EXEMPLARY FIRST ORGANIC SOLVENT MICROCAPSULE FORMULATIONS

5 The following formulations have been used with particular success by the inventors in both earth normal and microgravity methods of making microcapsules.

Fluid 1 - (hydrocarbon). The first solvent is a hydrocarbon fluid (ethyl alcohol, methyl alcohol, or isopropyl alcohol) with a low or medium HLB (HLB=5-10). One or more co-solvents are used (which also can act as co-surfactants). Small concentrations of oil and water are added. Into this mixture, the mono- or polyglyceride is dissolved up to 5% w/v. An example is:

15 88% IPA
 2.5% m-Hexanol
 2.5% n-Heptanol
 5% IPO
 2% H₂O
20 5% GMS

Fluid 2 (aqueous). The second solvent is water plus surfactants (ex. polyethoxylated sorbitan esters; polyethylene glycol). A polysaccharide (Dextran) and normal saline (0.9%) are added which helps achieve the desired critical micelle concentration. A pharmaceutical soluble in water is added. An example is:

 1% PEG 4000
 5% Dextran-40 (MW=40,000)
 0.9% Sodium chloride

2% Sorbitan Monooleate/20 moles Ethylene oxide
Water (up to 100% volume)
dissolved drug at specified concentration
(according to required dose and release rate)

5 **Fluid 3 (oil)**. An oil, immiscible with the first two fluids
in which the microcapsule's "outer skin" is insoluble so
that the suspended microcapsules can be delivered by
injection when non-aqueous administration is required.
Submersion of microcapsules in the oil also can aid the
10 curing or polymerization of the "outer skin." A preferred
example of the oil vehicle is iodinated poppy seed oil which
also serves as a radiocontrast medium.

Alternate compositions for Fluid 1

Main solvent - ethyl alcohol

15 Co-solvents - (co-surfactants) are normal alcohols - C4 to
C8

high dielectric constant solvents -

tetrahydrofuran

dioxane

20

acetonitrile

dimethylformamide

dimethylacetamide

dimethylsulfoxide

- Oil - dense radiocontrast liquids s.a. iodinated
unsaturated oils
e.g. poppy seed oil, cotton seed oil, safflower
oil, olive oil, canola oil, peanut oil, sesame
oil, corn oil.
also saturated oils can be used, s.a. heavy
mineral oil, liquid petrolatum
- Polymers - used to form the "outer skin" on the
microcapsules monoglycerides - esp. glycerol
esters ranging from C12 - C22,
e.g. monostearate, distearates, monooleates,
monolaurates and olive oil
polyglycerides - cholesterol, waxy plant sterols
(stigmasterol, phytosterol, campesterol)
phospholipids - lecithins (phosphatydyl choline)
and/or combinations with mono/polyglycerides

Alternate concentrations:

<u>Fluid 1:</u>	Main solvent	75-95%
	Co-solvents	1-10%
	Oil	1-10%
	Polymer	1-5%
	Water	1-20%

Alternate composition for Fluid 2

- PEG 200-10000
Dextran-40 (MW = 40,000-70,000)
0.9% Sodium chloride
Sorbitan Monolaurate/20 moles Ethylene oxide

balance is water

Drug dissolved at saturated or specified
concentration

(according to required dose and release rate)

5 **Alternate concentrations:**

PEG	1-5%
Dextran (MW=40,000-70,000)	5-10%
Sodium chloride	0.9%
Sorbitan Monolaurate/20ETO	1-5%

10

Water (balance of volume)

Drug concentration saturated or specified

Alternate composition for Fluid 3 (Oils)

Dense radiocontrast liquids s.a. iodinated unsaturated
oils e.g. poppy seed oil, cotton seed oil, safflower oil,
15 olive oil, canola oil, peanut oil, sesame oil, corn oil.

Also saturated oils can be used, s.a. heavy mineral oil

Alternate concentrations: 100% oil or a mixture is used as
a carrier vehicle for the suspended microcapsules

20 **Example X:** EXEMPLARY FIRST AQUEOUS SOLVENT MICROCAPSULE
FORMULATIONS

ALTERNATE METHOD - Hydrophilic Outer Skin

Fluid 1 - (aqueous); the main solvent is a water, one or
more co-solvents (which also can act as co-surfactants), and
a lecithins is dissolved up to 5% w/v to form the outer skin
25 on the microcapsules.

REFERENCES CITED

The following references to the extent that they provide procedural details supplementary to those set forth herein, are specifically incorporated herein by reference.

- 5 Allen, T. M.. Interactions of Drug Carriers with the Mononuclear Phagocytic System, in G. Gregoriadis (Ed.) Liposomes as Drug Carriers, John Wiley & Sons Ltd., New York, pp.37-50, 1988.
- Allen, T. M., Mehra, T., Hansen, C. and Chin, Y.C., Stealth
10 Liposomes: An Improved Sustained Release System for 1-b-D-Arabinofuranosylcytosine, Cancer Res. 52:2431-39, 1992.
- Bhargava, H. N., Narurkar, A., and Lieb, L. M., Using
15 Microemulsions for Drug Delivery, Pharmaceutical Technology, pp. 46-54, March 1987.
- Gabizon, A., et al., Liposome-Associated Doxorubicin: Preclinical Pharmacology and Exploratory Clinical Phase, in G. Lopez-Berestein and I.J. Fidler (Eds.) Therapy of Infectious Diseases and Cancer, Alan R. Liss, Inc., New
20 York, pp. 189-203, 1992.
- Halbert, G.W. , Stuart, J.B., Florence, A.T., The Incorporation of Lipid Soluble Antineoplastic Agents into Microemulsions-Protein-free Analogues of Low Density Lipoprotein, Int. J. Pharm. 21: 219-232, 1984.

Kimler, B. F, et al., Combination of Aziridinylbenzoquinone and Cis-platinum with Radiation Therapy in the 9L Rat Brain Tumor Model, Int. J. Radiation Oncology Biol. Phys, 26: 445 - 450, 1993.

5 McCutcheon's Detergents and Emulsifiers, 1979, North American Edition, McCutcheon Division, MC Publishing Co., 175 Rock Road, Glen Rock, NJ 07452.

Parikl, I. and Stern, W. Microcrystal™ Drug Delivery System, in Harvey S. Price (Ed) The Biotechnology Report 1993/94,
10 Bookbuilders, Ltd., Hong Kong, pp. 219-220, 1994.

Talsma, H. and Crommelin, D. J. A., Liposomes as Drug Delivery Systems, Part 1: Preparation. Pharmaceutical Technology, pp. 96-106, October 1992.

Wright, K.C., Wallace, S., Mosier, B. and Mosier, D.,
15 Microcapsules for Arterial Chemoembolization: Appearance and In Vitro Drug Release Characteristics, J. Microencapsulation 5:13-20, 1988.

* * * * *

The present invention has been described in terms of
20 particular embodiments found or proposed to comprise preferred modes for the practice of the invention. It will be appreciated by those of skill in the art that, in light of the present disclosure, numerous modifications and changes can be made in the particular embodiments
25 exemplified without departing from the intended scope of the invention. For example, one alternate embodiment includes use of aqueous-soluble cyclodextrin (in the hydrophilic

phase) which has hydrophobic center that can itself entrap hydrophobic drugs, thereby acting as a carrier for hydrophobic drugs within the aqueous phase. Another alternate embodiment allows after microcapsules are formed, 5 for ancillary polymeric outer coats to be applied by conventional methods (electrostatic coating, aerosolization and drying, etc.). This is made possible by designing the precise chemical makeup of the initial polymeric outer skin such that it will be compatible with both drug diffusion and 10 the ancillary coating to be applied. When surfactants are used to facilitate adhesion of the third solution or ancillary coating the HLB must be selected to be compatible with the HLB of the existing outer coating which has already been formed, such that the solution containing the ancillary 15 coating will wet the surface of the existing outer coating, to enable deposition of the ancillary coating. This is in contrast to conventional liposomes whose outer membrane composition is a variable, depending on the phase separation of the phospholipids and cholesterol adduct when each 20 liposome forms. Another alternative embodiment incorporates an energy absorbing medium (e.g. photoactivator) which can absorb electromagnetic, ultraviolet, infrared, ultrasonic, radiofrequency and microwave radiation and thereby cause activation of a short-lived drug component just prior to 25 administration or after the microcapsules have reached the target site. Another embodiment incorporates magnetic particles and magnetic fields or free-fluid electrophoretic mechanisms, etc. to facilitate dispersion or transport of one phase across the immiscible interface into the other 30 phase. This has been demonstrated as a single pass, uni-directional form of mixing that is best exploited in

microgravity. Another embodiment includes attachment of certain (hydrophobic) antibodies to the polymeric skin which gives the microcapsules site specificity by being able to bind to target cells (e.g. tumor) while entrapped drugs
5 diffuse out to provide maximum doses to those cells with that antigenic site. Another embodiment makes use of polyethylene glycol (PEG) complexed to peptide or protein drugs and a customized polymeric outer skin which permits the drug-PEG complex to diffuse out of the microcapsule as
10 an intact entity. This permits the drug to resist antibody attachment and remain in the blood stream longer as found in the Pegnology SM type of drug complexes developed by Enzon, Inc. The improvement being delivery of this complex in the tailored microcapsules and controlled release of the
15 complex through the specially designed polymeric outer skin. All such modifications are intended to be included within the scope of the appended claims.

WHAT IS CLAIMED IS:

- 1 1. A method of making a multi-layered microcapsule,
2 comprising:
3 formulating a first phase comprising a first
4 solvent, a first polymer soluble in said first phase,
5 oil, and water;
6 formulating a second phase immiscible with said
7 first phase, said second phase comprising a second
8 solvent, a second polymer soluble in said second phase,
9 a surface active agent, and a salt;
10 said surface active agent having a
11 hydrophilic/lipophilic balance value greater than that
12 of said first polymer;
13 said second polymer having a
14 hydrophilic/lipophilic balance value lower than that of
15 said surface active agent;
16 creating an interface between said first and
17 second phases in a manner that limits fluid shear; and,
18 maintaining adsorptive surface characteristics at
19 said interface.
- 1 2. The method of claim 1 wherein said first solvent is
2 organic.
- 1 3. The method of claim 2 wherein said organic solvent is
2 selected from the group of organic solvents consisting
3 of methyl alcohol, ethyl alcohol, and isopropyl
4 alcohol.

- 1 4. The method of claim 1 wherein said first polymer is
2 selected from the group of polymers consisting of
3 glycerol monostearate, glycerol monooleate, glycerol
4 monolaurate, glycerol dioleate, glycerol distearate,
5 cholesterol, stigmasterol, phytosterol, campesterol,
6 and lecithins.
- 1 5. The method of claim 1 wherein said first solvent is
2 aqueous.
- 1 6. The method of claim 5 wherein said first polymer is
2 selected from the group of polymers consisting of
3 polyvinyl pyrrolidone, polyvinyl alcohols,
4 hydrocolloids, and lecithins.
- 1 7. The method of claim 6 wherein said hydrocolloid is
2 selected from the group of hydrocolloids consisting of
3 gelatin, gum tragacanth, carrageenan, Karaya gum, Guar
4 gum, gum arabic, alginates, carboxymethyl cellulose,
5 hydroxypropyl cellulose, hydroxy propyl cellulose, and
6 carboxypropyl cellulose.
- 1 8. The method of claim 1, said first phase further
2 comprising a co-solvent, wherein said co-solvent is
3 selected from the group of co-solvents consisting of
4 C₃-C₈ alcohols, tetrahydrofuran, dioxane, acetonitrile,
5 dimethylformamide, dimethylacetamide, and dimethyl
6 sulfoxide.
- 1 9. The method of claim 1 wherein said oil is selected from
2 the group of oils consisting of poppy seed oil, olive

3 oil, peanut oil, sesame oil, cotton seed oil, soybean
4 oil, safflower oil, corn oil, sunflower seed oil,
5 canola oil, mineral oil, long chain paraffinic oil, and
6 liquid petrolatum.

1 10. The method of claim 1 wherein said oil is an
2 unsaturated oil which has been halogenated.

1 11. The method of claim 1 wherein said second polymer is
2 selected from the group of polymers consisting of
3 polyethyleneglycol 1000-8000 daltons, dextran 1000-
4 10000 daltons, polyvinylpyrrolidone, polyvinyl
5 alcohols, and hydrocolloids.

1 12. The method of claim 11 wherein said hydrocolloid is
2 selected from the group of hydrocolloids consisting of
3 gelatin, gum tragacanth, carrageenan, Karaya gum, Guar
4 gum, gum arabic, alginates, carboxymethyl cellulose,
5 hydroxypropyl cellulose, and carboxypropyl cellulose.

1 13. The method of claim 1 wherein said surface active agent
2 has a hydrophilic/lipophilic balance value of about
3 10.0 or greater.

1 14. The method of claim 13 wherein said surface active
2 agent is selected from the group of surface active
3 agents consisting of sorbitan monooleate treated with
4 ethylene oxide, dextran, polyethylene glycol, C₁₂-C₂₀
5 fatty acids, 2-amino-2-methyl-1-propyl aminomethyl
6 propanol, amphoteric salts, and quaternary ammonium
7 salts.

- 1 15. The method of claim 1 wherein said salt is selected
2 from the group of salts consisting of NaCl, KCl, CaCl₂,
3 MgCl₂, quaternary ammonium salts, cetyl
4 trimethylammonium bromide, and 4-methoxy-4(3-
5 phosphatidyl choline)spiro(1,2-dioxetane-3-g,1-
6 adamantane) disodium salt.
- 1 16. The method of claim 1 wherein said first phase further
2 comprises a pharmaceutical composition.
- 1 17. The method of claim 16 wherein said pharmaceutical
2 composition is selected from the group of
3 pharmaceutical compositions consisting of cytotoxins,
4 proteases, cytokines, anti-nauseants, steroids, anti-
5 fungal agents, fibrinolytic enzymes, and antibiotics.
- 1 18. The method of claim 16 wherein said pharmaceutical
2 composition is in suspension.
- 1 19. The method of claim 1 wherein said second phase further
2 comprises a pharmaceutical composition.
- 1 20. The method of claim 19 wherein said pharmaceutical
2 composition is selected from the group of
3 pharmaceutical compositions consisting of cytotoxins,
4 proteases, cytokines, anti-nauseants, steroids, anti-
5 fungal agents, fibrinolytic enzymes, and antibiotics.
- 1 21. The method of claim 19 wherein said pharmaceutical
2 composition is in suspension.

- 1 22. The method of claim 16 or 19 wherein said
2 pharmaceutical is at a concentration sufficient to
3 allow nascent crystal formation within said
4 microcapsule.
- 1 23. The method of claim 1, wherein after said microcapsule
2 forms, taking additional steps comprising:
3 formulating a third phase comprising an oil or
4 C₂₀-C₃₈ paraffin; and,
5 contacting said microcapsule with said third
6 phase.
- 1 24. The method of claim 23, wherein said third phase
2 further comprises a polymer.
- 1 25. The method of claim 23, wherein said third phase
2 further comprises a pharmaceutical composition.
- 1 26. The method of claim 1, wherein after said microcapsule
2 forms, taking additional steps comprising:
3 formulating a third phase comprising an aqueous
4 solution; and,
5 contacting said microcapsule with said third
6 phase.
- 1 27. The method of claim 26, wherein said third phase
2 further comprises a polymer.
- 1 28. The method of claim 26, wherein said third phase
2 further comprises a pharmaceutical composition.

- 1 29. The method of claim 26, wherein said third phase
2 further comprises an adjuvant.
- 1 30. The method of claim 26, wherein said third phase
2 further comprises an activator agent.
- 1 31. The method of claim 29, wherein said adjuvant further
2 comprises an immunoglobulin, peptide, protein,
3 hydrocolloid or polysaccharide.
- 1 32. The method of claim 31, wherein said hydrocolloid is
2 selected from the group of such hydrocolloids
3 consisting of collagen, isoelectric gelatin, agar, gum
4 arabic, gum tragacanth, cellulose derivatives,
5 alginates and carrageenans.
- 1 33. The method of claim 26, wherein said third phase
2 further comprises a surface active agent compatible
3 with the hydrophilic/lipophilic balance value of an
4 outer coating of said microcapsule.
- 1 34. The method of claim 1 wherein one or more of said
2 phases further comprise fluorescent molecules selected
3 from the group of fluorescent molecules consisting of
4 fluoresceins, cyanins, naturally fluorescent molecules,
5 and rhodamines.
- 1 35. The method of claim 1 wherein said fluid shear is
2 limited to a force of less than about 12 dynes per
3 square centimeter at said interface.

- 1 36. The method of claim 1 wherein said adsorptive surface
2 characteristics comprise a Helmholtz charge
3 distribution at said interface.
- 1 37. The method of claim 1 wherein said maintenance of
2 adsorptive surface characteristics is promoted by
3 carrying out the method under microgravity.
- 1 38. The method of claim 37 wherein said microgravity is at
2 least 1.0 minute in duration.
- 1 39. The method of claim 1 wherein said maintenance of
2 adsorptive surface characteristics is promoted by
3 carrying out the method below ambient temperature.
- 1 40. The method of claim 1 wherein said maintenance of
2 adsorptive surface characteristics is promoted by
3 substantially balancing the specific gravity between
4 said phases.
- 1 41. A method of making a multi-layered microcapsule,
2 comprising:
3 formulating a first phase comprising an organic
4 solvent selected from the group of organic solvents
5 consisting of methyl alcohol, ethyl alcohol and
6 isopropyl alcohol, a first polymer soluble in said
7 first phase selected from the group of polymers
8 consisting of glycerol monostearate, glycerol
9 monooleate, glycerol monolaurate, glycerol dioleate,
10 glycerol distearate, cholesterol, stigmasterol,
11 phytosterol, campesterol, and lecithins, a co-solvent

12 selected from the group of co-solvents consisting of
13 C₃-C₈ alcohols, tetrahydrofuran, dioxane, acetonitrile,
14 dimethylformamide, dimethylacetamide, and dimethyl
15 sulfoxide, an oil selected from the group of oils
16 consisting of poppy seed oil, olive oil, peanut oil,
17 sesame oil, cotton seed oil, soybean oil, safflower
18 oil, corn oil, sunflower seed oil, canola oil, mineral
19 oil, long chain paraffinic oil, and liquid petrolatum,
20 and water;

21 formulating a second phase immiscible with said
22 first phase, said second phase comprising water, a
23 second polymer soluble in said second phase selected
24 from the group of polymers consisting of
25 polyethyleneglycol 1000-8000 daltons, dextran 1000-
26 10000 daltons, polyvinylpyrrolidone, polyvinyl
27 alcohols, hydrocolloids, and lecithins, a surface
28 active agent selected from the group consisting of
29 sorbitan monooleate treated with ethylene oxide,
30 dextran, polyethylene glycol, C₁₂-C₂₀ fatty acids, 2-
31 amino-2-methyl-1-propyl aminomethyl propanol,
32 quaternary ammonium salts, and a salt selected from the
33 group of salts consisting of NaCl, KCl, CaCl₂, MgCl₂,
34 quaternary ammonium salts, cetyl trimethylammonium
35 bromide, and 4-methoxy-4(3-phosphatidyl
36 choline)spiro(1,2-dioxetane-3-g,1-adamantane) disodium
37 salt;

38 said surface active agent having a hydrophilic/
39 lipophilic balance value greater than that of said
40 first polymer;

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41 said second polymer having a
42 hydrophilic/lipophilic balance value lower than that of
43 said surface active agent;
44 creating an interface between said first and
45 second phases in a manner that substantially limits
46 fluid shear; and,
47 substantially maintaining adsorptive surface
48 characteristics at said interface.

1 42. Microcapsules produced by any of the methods of the
2 preceding claims.

1 43. The microcapsules of claim 42 wherein said
2 microcapsules are of a uniform distribution of
3 diameters.

1 44. The microcapsules of claim 43 wherein said diameter
2 distribution is in a range of about 1.0 to 350.0
3 microns.

1 45. The microcapsule of claim 42 further comprising at
2 least one nascent crystal of a pharmaceutical
3 composition.

1 46. The microcapsules of claim 45 wherein said crystal
2 occupies at least about 10 percent of the volume of
3 said microcapsule.

1 47. A microcapsule produced by the method of claim 41.

1 48. A multi-layered microcapsule, comprising:

2 a first layer comprising a first solvent, a first
3 polymer soluble in said first layer, oil, and water;
4 a second layer immiscible with said first layer,
5 said second layer comprising a second solvent, a second
6 polymer soluble in said second layer, a surface active
7 agent, and a salt;
8 said surface active agent having a hydrophilic/
9 lipophilic balance value greater than that of said
10 first polymer; and,
11 said second polymer having a
12 hydrophilic/lipophilic balance value lower than that of
13 said surface active agent.

1 49. The microcapsule of claim 48 wherein said first solvent
2 is organic.

1 50. The microcapsule of claim 49 wherein said organic
2 solvent is selected from the group of organic solvents
3 consisting of methyl alcohol, ethyl alcohol and
4 isopropyl alcohol.

1 51. The microcapsule of claim 49 wherein said first polymer
2 is selected from the group of polymers consisting of
3 glycerol monostearate, glycerol monooleate, glycerol
4 monolaurate, glycerol dioleate, glycerol distearate,
5 cholesterol, stigmasterol, phytosterol, campesterol,
6 and lecithins.

1 52. The microcapsule of claim 48 wherein said first solvent
2 is aqueous.

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- 1 53. The microcapsule of claim 52 wherein said first polymer
2 is selected from the group of polymers consisting of
3 polyvinyl pyrrolidone, polyvinyl alcohols,
4 hydrocolloids, and lecithins.
- 1 54. The microcapsule of claim 53 wherein said hydrocolloid
2 is selected from the group of hydrocolloids consisting
3 of gelatin, gum tragacanth, carrageenan, Karaya gum,
4 Guar gum, gum arabic, alginates, carboxymethyl
5 cellulose, hydroxypropyl cellulose, and carboxypropyl
6 cellulose.
- 1 55. The microcapsule of claim 48, said first layer further
2 comprising a co-solvent, wherein said co-solvent is
3 selected from the group of co-solvents consisting of
4 C₃-C₈ alcohols, tetrahydrofuran, dioxane, acetonitrile,
5 dimethylformamide, dimethylacetamide, and dimethyl
6 sulfoxide.
- 1 56. The microcapsule of claim 48 wherein said oil is
2 selected from the group of oils consisting of poppy
3 seed oil, olive oil, peanut oil, sesame oil, cotton
4 seed oil, soybean oil, safflower oil, corn oil,
5 sunflower seed oil, canola oil, mineral oil, long chain
6 paraffinic oil, and liquid petrolatum.
- 1 57. The microcapsule of claim 48 wherein said oil is an
2 unsaturated oil which has been halogenated.
- 1 58. The microcapsule of claim 48 wherein said second
2 polymer is selected from the group of polymers

3 consisting of polyethyleneglycol 1000-8000 daltons,
4 dextran 1000-10000 daltons, polyvinylpyrrolidone,
5 polyvinyl alcohols, gelatin, gum tragacanth,
6 carrageenan, Karaya gum, Guar gum, gum arabic,
7 alginates, carboxymethyl cellulose, hydroxypropyl
8 cellulose, carboxypropyl cellulose, and lecithins.

1 59. The microcapsule of claim 48 wherein said surface
2 active agent has a hydrophilic/lipophilic balance value
3 of about 10.0 or greater.

1 60. The microcapsule of claim 59 wherein said nonionic
2 surface active agent is selected from the group of
3 nonionic surface active agents consisting of sorbitan
4 monooleate treated with ethylene oxide, dextran,
5 polyethylene glycol, C₁₂-C₂₀ fatty acids, 2-amino-2-
6 methyl-1-propyl aminomethyl propanol, amphoteric salts,
7 and quaternary ammonium salts.

1 61. The microcapsule of claim 48 wherein said salt is
2 selected from the group of salts consisting of NaCl,
3 KCl, CaCl₂, MgCl₂, quaternary ammonium salts, cetyl
4 trimethylammonium bromide, and 4-methoxy-4(3-
5 phosphatidyl choline)spiro(1,2-dioxetane-3-g,1-
6 adamantane) disodium salt.

1 62. The method of claim 48 wherein said first layer further
2 comprises a pharmaceutical composition.

1 63. The microcapsule of claim 62 wherein said
2 pharmaceutical composition is selected from the group

3 of pharmaceutical compositions consisting of
4 cytotoxins, proteases, cytokines, anti-nauseants,
5 steroids, anti-fungal agents, fibrinolytic enzymes, and
6 antibiotics.

1 64. The microcapsule of claim 62 wherein said
2 pharmaceutical composition is in suspension.

1 65. The microcapsule of claim 48 wherein said second layer
2 further comprises a pharmaceutical composition.

1 66. The microcapsule of claim 65 wherein said
2 pharmaceutical composition is selected from the group
3 of pharmaceutical compositions consisting of
4 cytotoxins, proteases, cytokines, anti-nauseants,
5 steroids, anti-fungal agents, fibrinolytic enzymes, and
6 antibiotics.

1 67. The microcapsule of claim 65 wherein said
2 pharmaceutical composition is in suspension.

1 68. The microcapsule of claim 62 or 65 wherein said
2 pharmaceutical is at a concentration sufficient to
3 allow nascent crystal formation within said
4 microcapsule.

1 69. The microcapsule of claim 48 comprising a third layer,
2 said third layer comprising an oil or paraffin.

1 70. The microcapsule of claim 69, wherein said third layer
2 further comprises a polymer.

- 1 71. The microcapsule of claim 69, wherein said third layer
2 further comprises a pharmaceutical composition.
- 1 72. The microcapsule of claim 69, wherein said third layer
2 further comprises a surface active agent.
- 1 73. The microcapsule of claim 48 comprising a third layer,
2 said third layer comprising an aqueous solution.
- 1 74. The microcapsule of claim 73, wherein said third layer
2 further comprises a polymer.
- 1 75. The microcapsule of claim 73, wherein said third layer
2 further comprises a pharmaceutical composition.
- 1 76. The microcapsule of claim 73, wherein said third layer
2 further comprises an adjuvant.
- 1 77. The microcapsule of claim 76, wherein said adjuvant
2 further comprises an immunoglobulin, peptide, protein,
3 hydrocolloid or polysaccharide.
- 1 78. The microcapsule of claim 77, wherein said peptide or
2 said protein selectively adheres to a target cell,
3 tissue or tumor.
- 1 79. The microcapsule of claim 77, wherein said hydrocolloid
2 is selected from the group of such hydrocolloids
3 consisting of collagen, isoelectric gelatin, agar, gum
4 arabic, gum tragacanth, cellulose derivatives and
5 carrageenans.

- 1 80. The microcapsule of claim 73, wherein said third layer
2 further comprises a surface active agent.
- 1 81. The microcapsule of claim 48 wherein one or more of
2 said layers further comprise fluorescent molecules
3 selected from the group of fluorescent molecules
4 consisting of fluoresceins, cyanins, naturally
5 fluorescent molecules, and rhodamines.
- 1 82. The microcapsule of claim 48 formed under microgravity.
- 1 83. The microcapsule of claim 82 wherein said microgravity
2 is at least 1.0 minutes in duration.
- 1 84. The microcapsule of claim 48 formed below ambient
2 temperature.
- 1 85. The microcapsule of claim 48 comprising layers at least
2 initially substantially balanced as to specific gravity
3 between said layers.
- 1 86. A multi-layered microcapsule, comprising:
2 a first layer comprising an organic solvent
3 selected from the group of organic solvents consisting
4 of methyl alcohol, ethyl alcohol and isopropyl alcohol,
5 a first polymer soluble in said first phase selected
6 from the group of polymers consisting of glycerol
7 monostearate, glycerol monooleate, glycerol monolaurate,
8 glycerol dioleate, glycerol distearate, cholesterol,
9 stigmasterol, phytosterol, campesterol, and lecithins,
10 a co-solvent selected from the group of co-solvents

11 consisting of C₃-C₈ alcohols, tetrahydrofuran, dioxane,
12 acetonitrile, dimethylformamide, dimethylacetamide, and
13 dimethyl sulfoxide, oil selected from the group of oils
14 consisting of poppy seed oil, olive oil, peanut oil,
15 sesame oil, cotton seed oil, soybean oil, safflower
16 oil, corn oil, sunflower seed oil, canola oil, mineral
17 oil, a long chain paraffinic oil, and liquid
18 petrolatum, and water;

19 a second layer immiscible with said first layer,
20 said second layer comprising water, a second polymer
21 soluble in said second phase selected from the group of
22 polymers consisting of polyethyleneglycol 1000-8000
23 daltons, dextran 1000-10000 daltons,
24 polyvinylpyrrolidone, polyvinyl alcohols, gelatin, gum
25 tragacanth, carrageenan, Karaya gum, Guar gum, gum
26 arabic, alginates, carboxymethyl cellulose,
27 hydroxypropyl cellulose, carboxypropyl cellulose, and
28 lecithins, a surface active agent sorbitan monooleate
29 treated with ethylene oxide, dextran, polyethylene
30 glycol, C₁₂-C₂₀ fatty acids, 2-amino-2-methyl-1-propyl
31 aminomethyl propanol, amphoteric salts, and quaternary
32 ammonium salts, and a salt selected from the group of
33 salts consisting of NaCl, KCl, CaCl₂, MgCl₂, quaternary
34 ammonium salts, cetyl trimethylammonium bromide, and 4-
35 methoxy-4(3-phosphatidyl choline)spiro(1,2-dioxetane-3-
36 g,1-adamantane) disodium salt;

37 said surface active agent having a
38 hydrophilic/lipophilic balance value greater than that
39 of said first polymer; and,

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40 said second polymer having a
41 hydrophilic/lipophilic balance value lower than that of
42 said surface active agent.

1 87. The microcapsule of claim 62 further comprising at
2 least two different pharmaceuticals, a first
3 pharmaceutical in said first phase and a second
4 pharmaceutical in said second phase.

1 88. The microcapsule of claim 48 further comprising a
2 radiopaque contrast agent.

1 89. The microcapsule of claim 48 further comprising a
2 magnetic resonance contrast agent.

1 90. The microcapsule of claim 89 wherein said contrast
2 agent is selected from the group of metallo-organic
3 compounds consisting of ferrous gluconate, Gadolinium
4 diethylenetriamine pentaacetic acid, and iron
5 pentacarbonyl.

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ABSTRACT

Methods of forming multi-lamellar microcapsules having alternating layers of hydrophilic and hydrophobic immiscible liquid phases have been developed using different

5 polymer/solvent systems. The methods use liquid-liquid diffusion and simultaneous lateral phase separation, controlled by proper timed-sequence exposures of immiscible phases and low shear mixing, to form narrow size distributions of spherical, multilamellar microcapsules.

10 The use of special formulations of solubilized drugs, surfactants, and polymeric co-surfactants in aqueous vehicles which are dispersed in hydrocarbon solvents containing small quantities of oil, low molecular weight co-surfactants and glycerides that are aqueous insoluble

15 enables the formation of unique microcapsules which can carry large amounts of pharmaceuticals in both aqueous and non-aqueous solvent compartments. The liquid microcapsules are quickly formed in a single step and can include a polymeric outer "skin" which protects the microcapsules

20 during physical manipulation or exposure to high shear forces. Water-in-oil and oil-in-water microcapsules have been formed both in 1 x g and in microgravity, which contain several types of drugs co-encapsulated within different fluid compartments inside the same microcapsule. Large,

25 spherical multi-lamellar microcapsules have been formed including a cytotoxic drug co-encapsulated with a radiocontrast medium which has advantages for chemoembolization of vascular tumors. In certain cases, crystals of the drug form inside the microcapsules providing

30 zero-order and first order, sustained drug release kinetics.

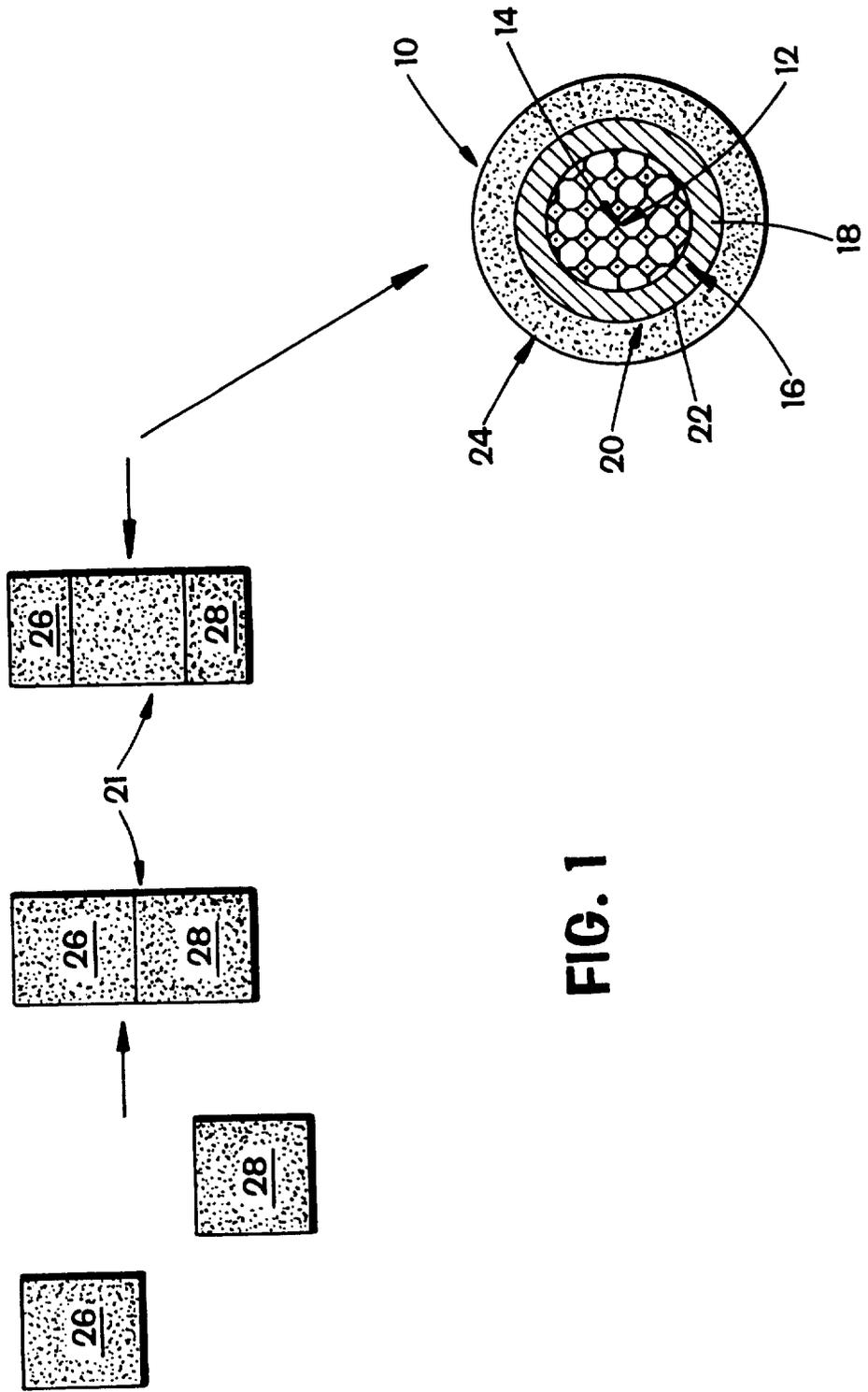


FIG. 1

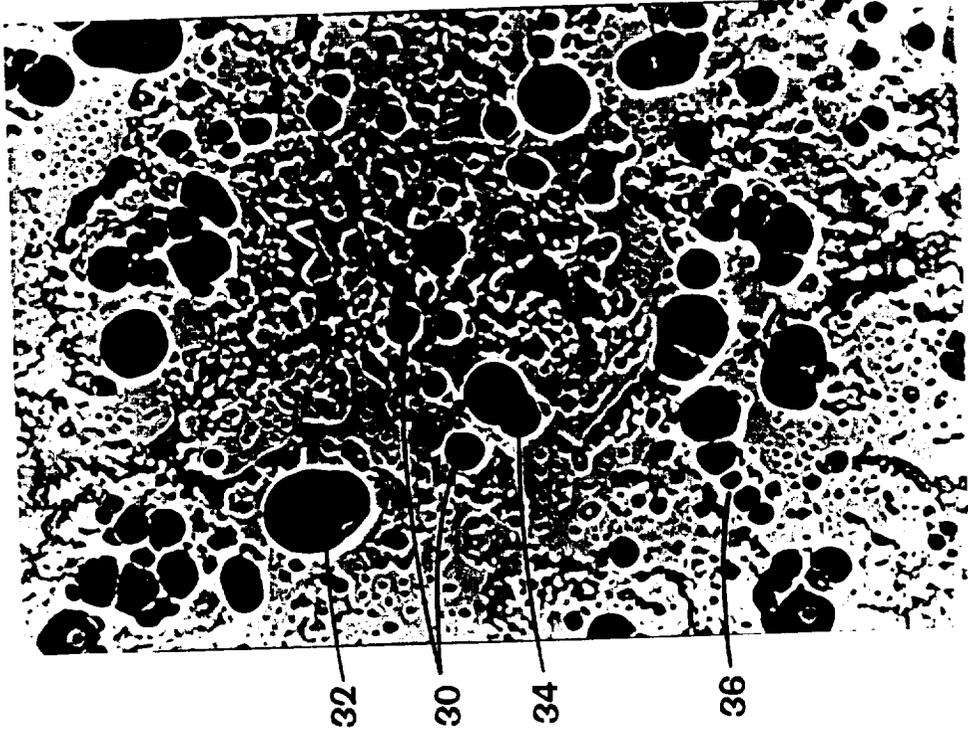


FIG. 2B

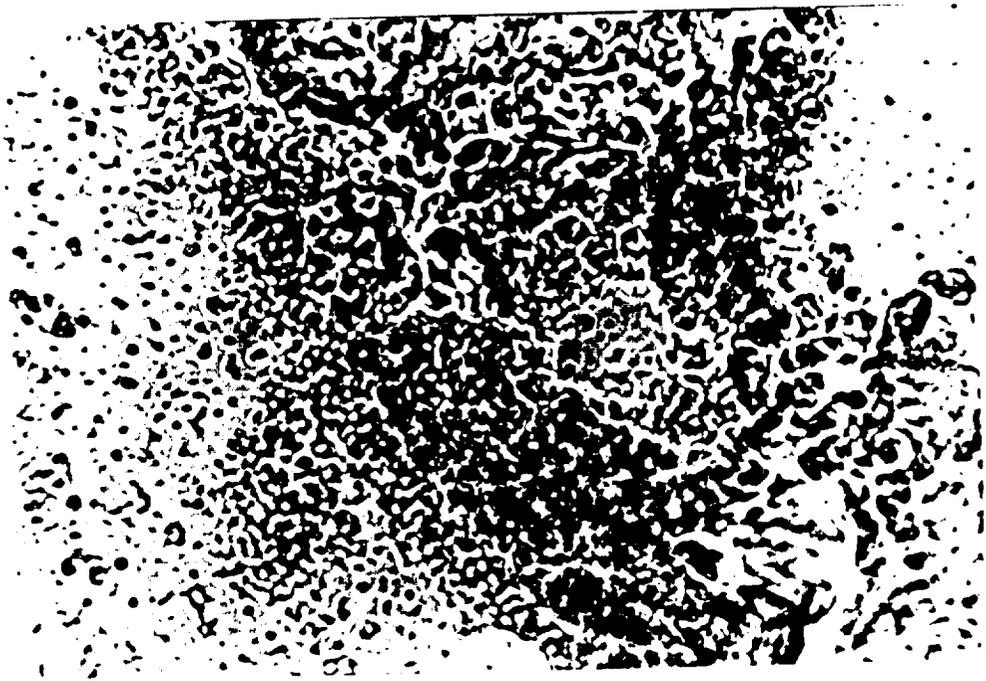


FIG. 2A

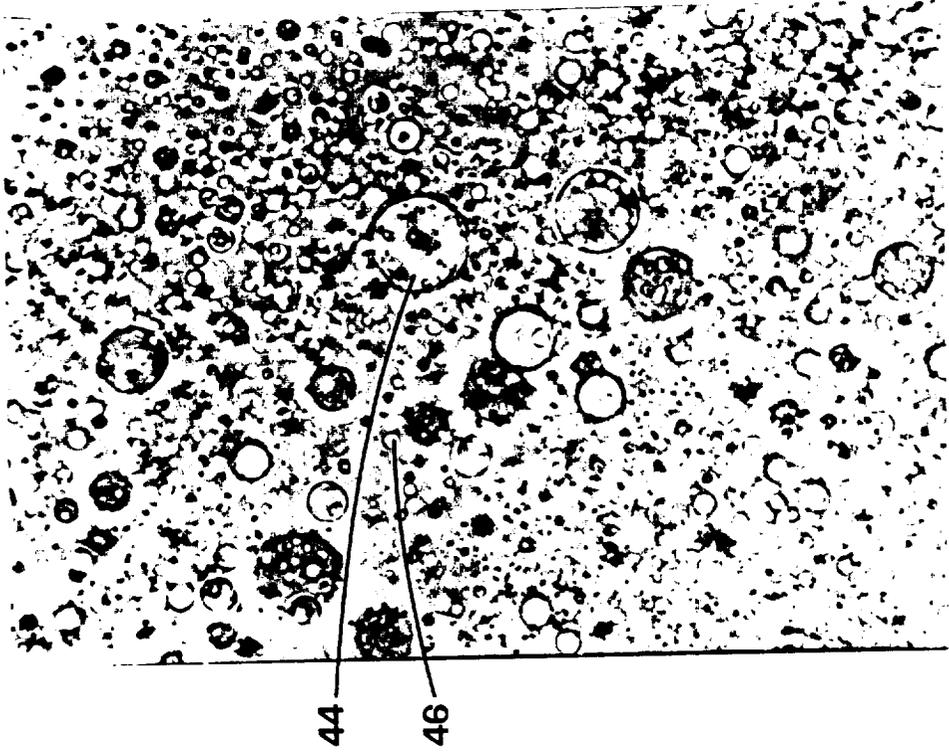


FIG. 3B

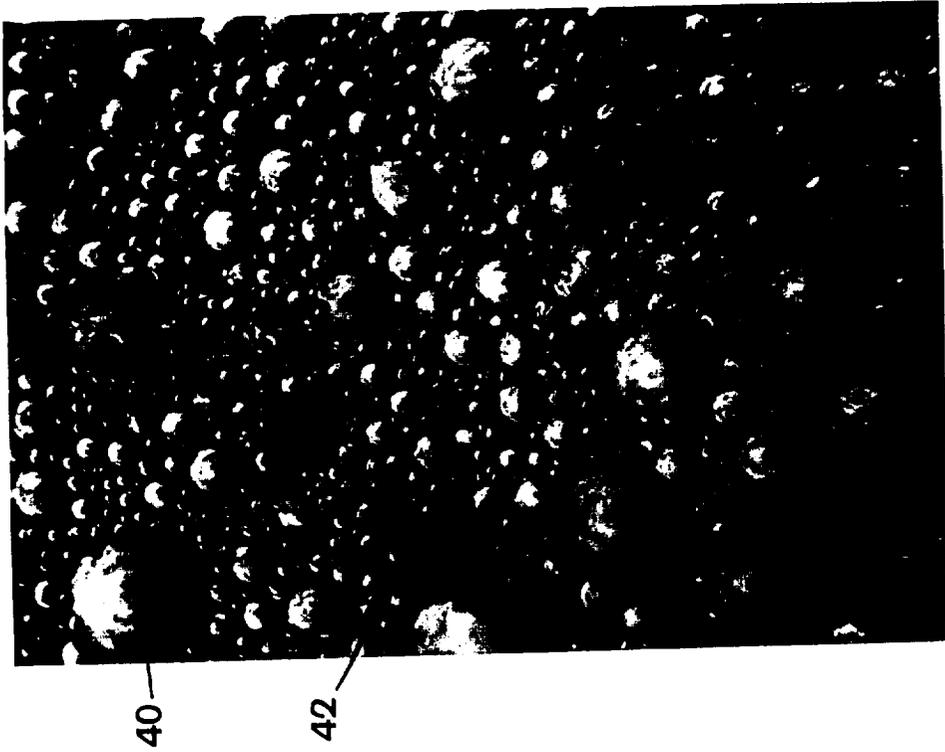


FIG. 3A

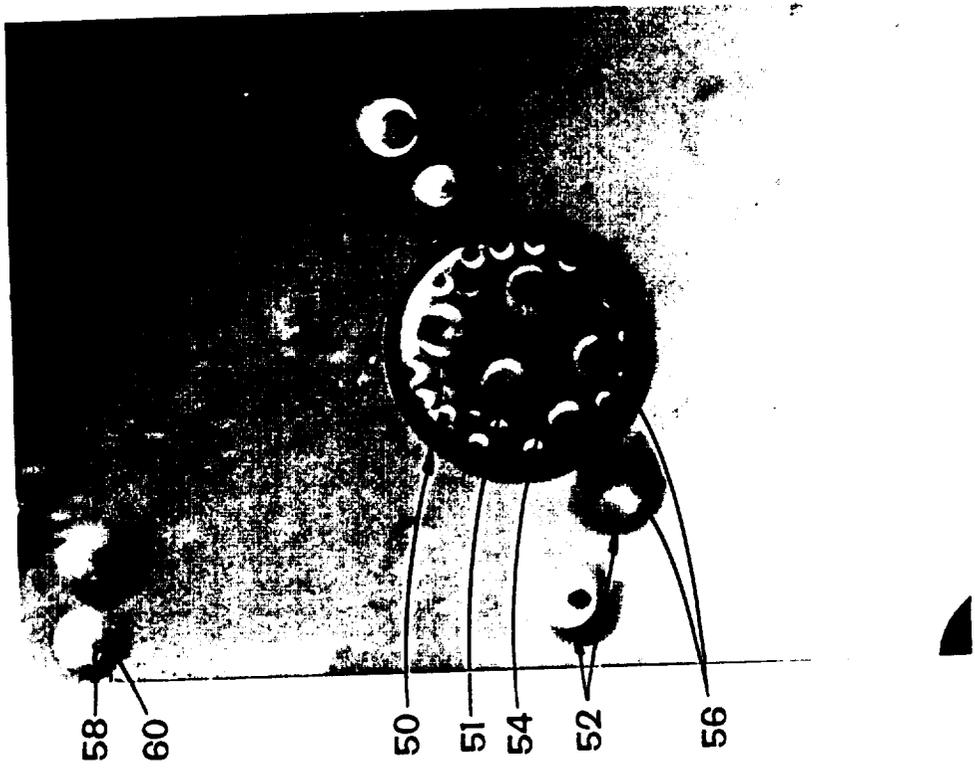


FIG. 4

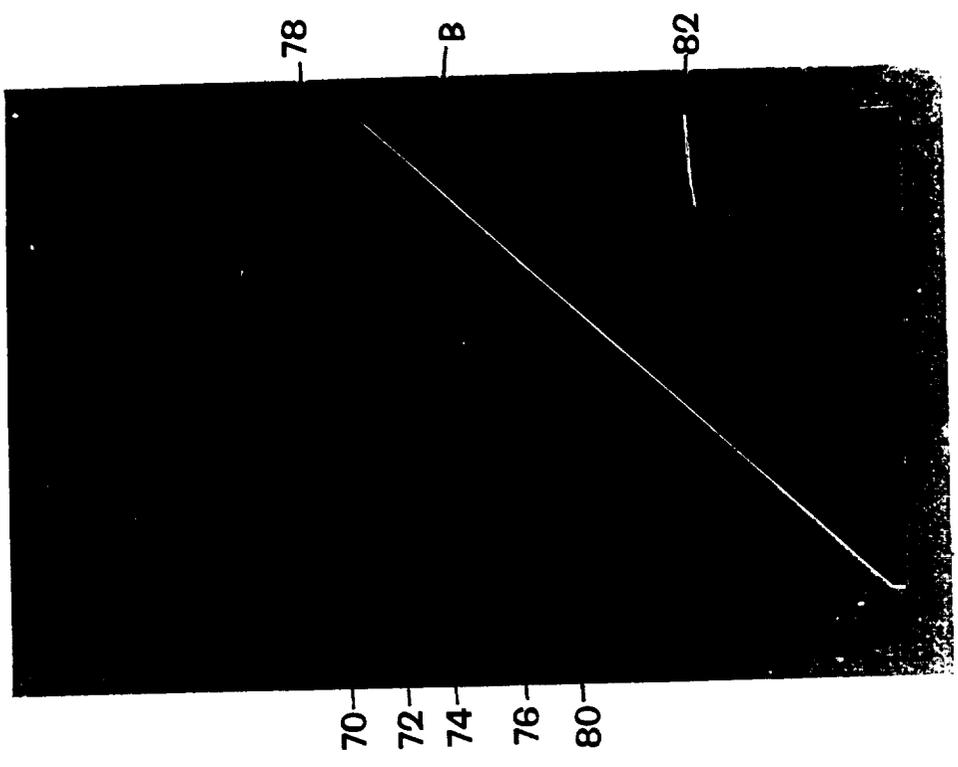


FIG. 5

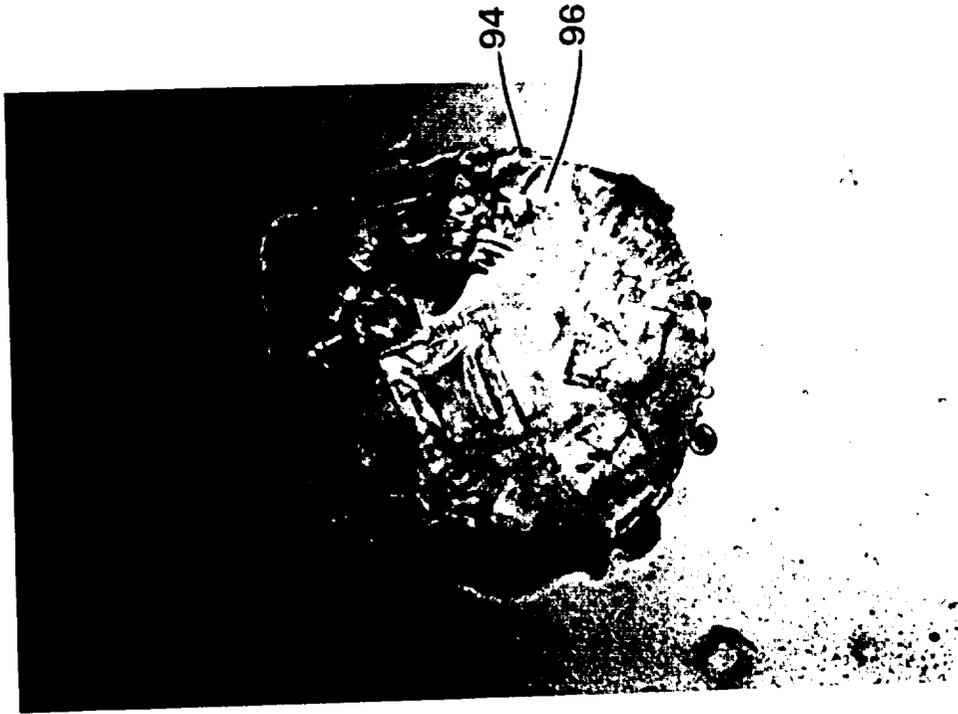


FIG. 6B

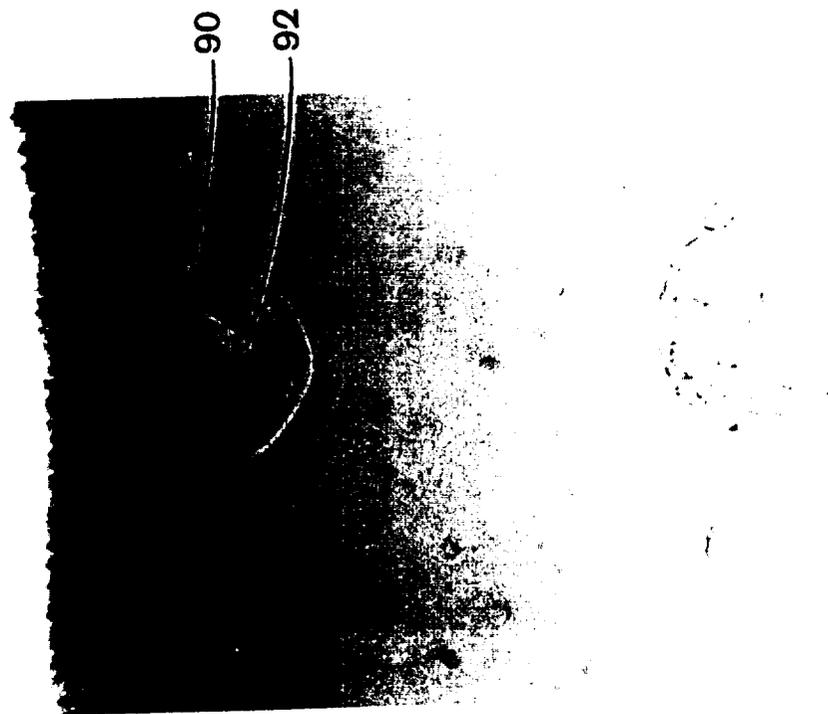


FIG. 6A

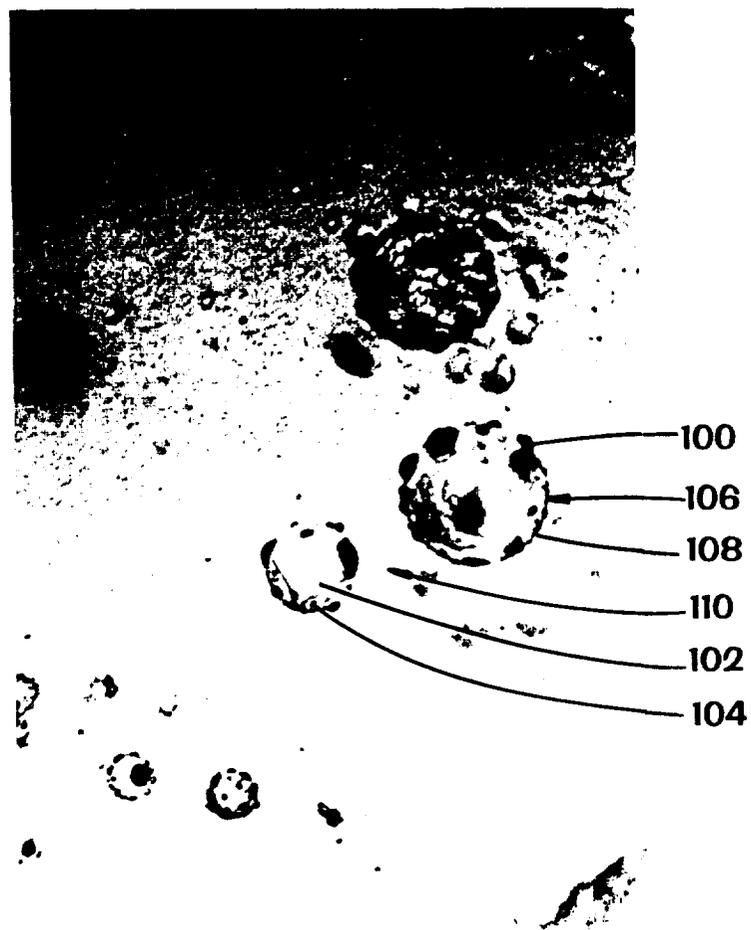


FIG. 7